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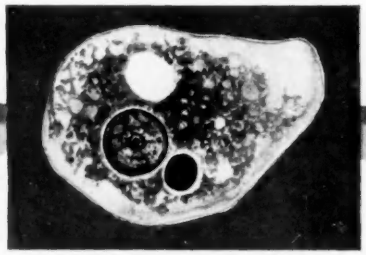

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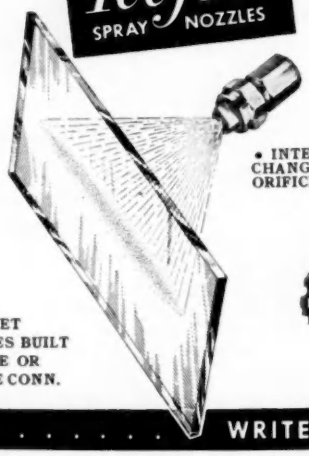

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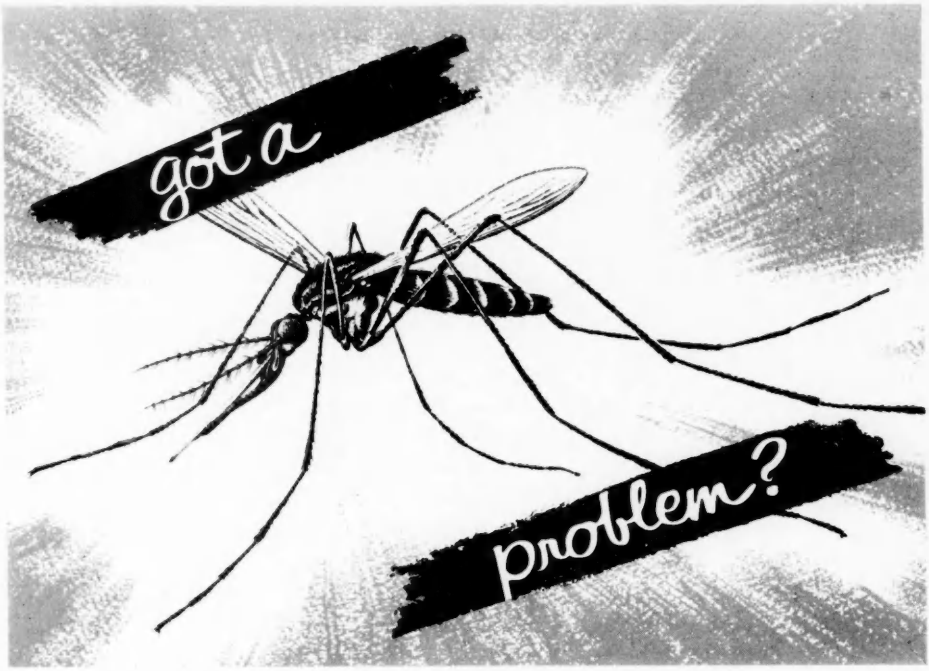

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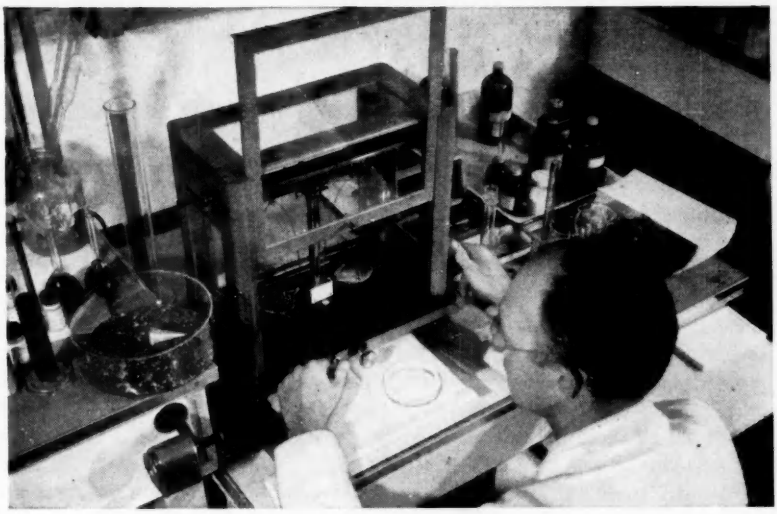

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OUR FUTURE?[1]

WM. A. SODEMAN[2]

Before proceeding with the substance of my discussion I want to express my sincere thanks for the honor and privilege of being entrusted with the guidance of the affairs of the Society for this past year. I am profoundly grateful.

In considering a subject for the presidential address, I, as I suppose have all presidents, considered what in my power I could do to make it a memorable address and a memorable occasion. Three important theoretical possibilities came to my mind. One, I could announce a very enlightening scientific study. Remember, I said theoretical possibilities. Two, there was the opportunity to develop a profound philosophical report which would seriously affect the future medical thought of all of you. Still theoretical! Thirdly, I could point out a satisfactory solution to the tangled problems of our own and related Societies. More theory! Finally there seemed to be a fourth and practical solution to make the occasion memorable. I could be brief! This approach was not only practical but also within my grasp. This approach I have chosen.

Preliminary to writing these words, again as I suppose many of the past presidents of our parent societies have done, I looked back upon previous presentations. A goodly number discussed our future. True, the terminology, varied. One time it was Horizons in Tropical Medicine or, after World War II, Tropical Medicine in Peace. Dr. Meleney's address this evening, entitled Unfinished Business, implies the same approach. Interestingly enough the discussions and thoughts varied widely. I find this true of my own presentation in comparing it with the others. Although the title is time-honored, the content I believe you will find no more repetitious than the remarks of preceding presidents.

I would like to point out that our society, as an organization, stems from an interested group of Philadelphia physicians, who, in 1903, organized the parent society of one of our parent societies (Faust, 1944). This group of men included internists, pathologists, neurologists, neuropsychiatrists, dermatologists, a surgeon, opthamologists, otolaryngologists, a therapeutist, and general practitioners. Although many of the group held outstanding professorial positions on the faculties of the medical schools in Philadelphia, none were experienced in the field of tropical medicine. They were all identified with other disciplines in medicine but professed an interest in learning about tropical disease. The diversified fields of basic medical interest represented in the founding group maintains itself in our society today. The great upsurge of interest in the preventive aspects of disease, together with the concomitant advancements, has added many others in categories of laboratory science and the preventive approach to the membership. Indeed the term "tropical medicine," in the minds

[1] Presidential Address given before the American Society of Tropical Medicine and Hygiene at Louisville, Kentucky, November 13, 1953.

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of most of us, now has the same connotation as the term "tropical health." The word medicine is used in the broad sense, as it is used in the name of a medical school. We all recognize as paramount in the tropics the major problems of disease control and health development and maintenance.

The point I want to make from the above discussion is this. We represent a cross section of the health profession, from clinicians and pathologists to epidemiologists, public health administrators, sanitarians and sanitary engineers, held together for varying reasons by interests that relate to health in tropical climes. What, in our future, is most important in maintaining and furthering these bonds? How can we effect an expanding interest and make greater strides in tropical health?

Tropical medicine itself, or indeed medicine generally, is not a science. The collection of facts as represented by the reports of our scientific program does not in itself make up a science. As those facts enter into the formulation and understanding of general laws of epidemiology or parasitology, for example, they lead to the organization and generalization of knowledge in those fields and produce the general laws which constitute a science. Medicine, and tropical medicine, will become sciences in their own right only after the collection of facts carries us to the establishment of basic laws of health and disease (Stevenson, 1949). We are not far on this road, a road which should represent our real future in tropical medicine. Our research is highly organized; our scientific achievements have been notable; our understanding of disease processes has increased immeasurably in recent years. But we lack proper advancement in the organization of this knowledge into important underlying principles. Some phases of our activity have advanced more remarkably than others along this road. Those interested in malaria can fittingly claim this honor to a greater degree than the rest of us. By calling themselves malariologists, even though they are epidemiologists, public health administrators, clinicians, and representatives of other basic scientific areas, those interested in this field give outward evidence of the coordinated effort they have developed. This cooperation in approaching the many facets of malaria had led to the synthesis of many of the facts and data relating to the various aspects of malaria into general laws which govern the development and spread not only of that disease but of other insect-borne diseases as well. Again much of this advancement is based upon the concept that a great reservoir of disease rests in animals other than man, and that the natural history of those diseases is governed in large part by the activities in that reservoir. This concept entails a coordination of effort on the total problem of the disease, upon the organism, the host *and* the environment. Indeed, the great advances in science have come not from the collection of new data, but from such correlation and the development of new concepts. Such basic laws we must seek. The success of our future in tropical medicine rests primarily upon our attempts to establish this goal.

What conditions are necessary to reach this goal? I think it is clear that one of the requirements is a greater interplay between those of us interested in our various basic fields. The highest development of specialism is not in medical

practice, but in research. One organism or group of organisms, one disease, or one organ, represents the interest of many of us. Under these conditions we may lose our insight into the total problem. Let me give you a minor but glaring example of inadequacies from such specialization. A short while ago, I saw a patient with hookworm disease and severe anemia. We treated the patient once and counted 2,000 worms in the stools. That sounded pretty good, generally. Doctor Paul Beaver had examined the original stool specimens and estimated from the eggs present that there were approximately 6,000 worms in the patient's bowel, indicating that our seemingly good results were really poor. A second round of treatment and still a third gave us enough worms to account for the 6,000 estimate, all together. Our medical students, impressed by the statement in their text that a hookworm can remove about 0.67 cc. or more of blood per worm per diem, indicated to us that the anemia resulted from the activity of worms carrying out this degree blood removal. If one knows the total blood volume of the average person, which is about 4–5,000 cc., he realizes that this situation in this patient would cause depletion of almost all the blood in the body in one day. Having discovered this through our prodding, the students then resorted to the additional statement in the text that it is likely that older worms produce considerably smaller blood loss, possibly not more than 0.1 cc. per worm daily, and calculated that this patient would lose at this figure 600 cc. per day, or the amount of a usual blood donation. One does not have to know anything about hookworms or the action of hookworms to know that these statements were not true in our patient. Laboratory investigation has indicated that a hookworm may remove blood to this degree, but not that all hookworms in a human infection are that efficient. Such statements represent a lack of correlation of information between the hemotologic physiologist and the parasitologist.

You might consider the example I have given as isolated and unimportant. I could multiply it many times if I wished, for it is a mere example of numerous instances of this type. It simply emphasizes the point of view that a collection of facts without organization and integration does not lead to a total understanding of the problem. The late Sir James Mackenzie, one of our greatest modern physicians and a clinician whom I have admired tremendously, wrote the same thing in different words when he said "for the intelligent practice of medicine and the understanding of disease, the simplication of medicine is necessary. . . . I hold that the phenomena which are at present so difficult of comprehension, on account of their number and diversity, are all produced in a few simple ways, and that with their recognition what is so complex and difficult will become simplified and easy to understand. This means a recognition of principles and a knowledge of their application" (Wolff, 1953).

The understanding of such principles must entail a cutting across the lines of various specialities and various disciplines. When this happens, whole new vistas open up which we did not appreciate before, and those of us with facts in our own little field begin to see ways to fit those facts into the general laws. An outstanding example of this is the stress and adaptation syndrome, in which a wealth of seemingly unrelated observations is integrated. Since man's adaptive

and protective capabilities are limited, his response to many sorts of noxious agents and threats may be similar, the form of the reaction to any one agent depending more on the individual's nature and past experience than upon the particular noxious agent evoking it, and this adaptive-protective reaction may be far more damaging to the individual than the effects of the noxious agent *per se*, be it direct effects of microorganisms, climate, physical forces, disruption of customs or habits, or others (Wolff, 1953). In the light of this concept, large groups of individuals, those interested in infections, the endocrinologists, cardiovascular specialists, pathologists, psychiatrists, all begin to find ways to fit the collection of facts in their own field into an organized system of understanding which governs events in all these fields. One can realize why chronic psychic trauma and chronic amebic infection might give the same symptoms.

If we are interested in *man* in the tropics, it is obvious that we must take the astounding advances in laboratory research on the agents of disease and the meagre gains in clinical observation in man and integrate them with each other and with the multitude of environmental factors playing on both. The one discipline which crosses all lines of activity, be it laboratory or field endeavor, is epidemiology. Knowledge obtained from the bedside study of the sick individual, and that of the controlled conditions in laboratory experimentation, both solid bases for modern medicine, requires, for thorough understanding of control and causation of disease, observations under natural conditions in the whole population. If epidemiology is called the natural history of disease, all medicine is no more than the natural history of disease with attempts to block it as early as possible. The clinical method and the experimental method are both then bound up with it. Encompassed in this scope, which we may broadly call medical ecology, is medical geography, a part and parcel of epidemiology. Those who profess to be epidemiologists, who are by no means all of those practicing or using epidemiology as you can see, have long recognized that infections are more than the interplay between the exciting agent and the host. The environment plays a profound role. Again the malariologists were early to recognize this, chiefly through study of the agent residing there. It is itself complex and many sided, with physical, biological, and social components (Gordon, 1952). As such, a disease is the result of no simple contest between the host and the agent of the disease. A great variety of environmental factors have a part in its origin, and a balance of affairs determines the issue. There can be no complete understanding of the process until the interplay of all three of these factors is evaluated. The laboratory investigator dealing with the agent studies but a fraction of the problem and collects facts which are inexplicable without knowledge of the host and environment. The clinician studying the host alone similarly reaches important limitations in his study.

All disease is of multiple causation. In some diseases in which a specific agent is not known in the causation, we study the multiple causes which are known very intensively and attempt to establish their relationship very extensively. This is true in peptic ulceration of the stomach or in high blood pressure. In disease entities in which we know the specific agent, there is a great tendency

to study the specific agent to the exclusion of the other etiologic factors. If we can isolate the specific agent, take it into the laboratory and manipulate it, we may of course learn very much about it and other causes, but we may be so interested in the agent that we study it to the exclusion of the other causes as well. We turn away from the relationship of host, agent, and environment. We are apt to study effect of environment on agent and not on host except for mechanism of transmission. But it is in the total relationships that we find factors of extreme importance. In pneumococcic pneumonia the agent may be in the throat of the individual but not produce the disease. It may take a common cold or an alcoholic bout to lay the ground work in the bronchioles to produce the disease entity. The common cold or the alcoholic bout are as important in etiologic relationships as is the pneumococcus itself in the production of disease in that patient. Similar factors are important in many of the diseases that we consider tropical. In amebic disease for example we find in some areas of the tropics when the cool season comes on, that amebic dysentery tends to disappear. The agent is there; the host is there; environment as a medium permitting spread of the agent has not changed remarkedly. Why has dysentery disappeared from the wards? The explanation most likely rests in host factors and can be explained by adequate study of the host. Increase in the host resistance of some sort or other is responsible for the reduction in the activity of the disease and the development of the low grade chronic disease with cystation. That is why I have always thought personally that the study of excystation and encystation is one of the most important in the field of amebiasis. It involves the relationship of man to the organism.

How little we know of man in his reactions and adaptation to tropical climate itself! The host is often little studied except as the agent relates to definite changes in him. And we cannot have a full understanding of his disease reactions until we gain such knowledge. Acute effects we know in some degree. But information on the more subtle changes of long term residence in the tropics is most fragmentary. We do know enough of the changes in thyroid, adrenal, and pituitary function to realize that widespread physiologic effects are at work, effects in and on organs and systems concerned with protective mechanisms, growth and metabolism, and reaction patterns. These modify responses to invasive agents and to therapeutic procedures. They may help explain, in part why clinical pictures differ in tropical and temperate practice, why drugs seem more effective in one part of the world than another, and why man fares better in one area than another. Indeed, they may help explain the modification in occurrence, as well as clinical activity, of some cosmopolitan diseases in the tropics and could lead to understanding of the processes involved, to discovery of facts helpful in the understanding, treatment, modification, and eradication of these "nontropical" diseases elsewhere in the world.

I have tried to use this discussion chiefly to emphasize the fact that specialism in study has great limitations. Specialized concentration on technics and fact finding in narrow fields have carried us far in our study of man in the tropics. But our ultimate goal is systemization and understanding of these facts, of

fitting them into a rational pattern. A broad concept of the disease, or of disease as a whole, must be stressed now and in the future. Our future rests in the development of more facts, that is true, in furtherance of the tremendously important laboratory investigations of the past, advancement in the field and clinical studies long under way. But it rests more importantly upon the correlation of the data obtained and to be obtained, and the synthesis of that information into an understanding of the underlying principles and general laws governing these processes. If our future is pointed toward that goal, the interests in tropical medicine of everyone in this room will be multiplied many times. The ties that bind us to tropical medicine, as workers and scientists in those fields which are the handmaidens of tropical medicine, will become ever stronger, and tropical medicine will be guided toward the achievement of stature as a science.

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PROBLEMS IN THE CONTROL OF SCHISTOSOMIASIS[1]

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Schistosomiasis has become recognized as ranking next to malaria in importance among the animal parasite diseases of man, when considered from the viewpoint of disability and deaths. Indeed, now that practical and economical methods of malaria control are available, schistosomiasis looms as the most important health problem to be solved in many tropical and subtropical regions. Stoll (1947) estimated that there were approximately 114 million cases of schistosomiasis in the world, representing about five per cent of the world's population.

The control of schistosomiasis is constantly receiving more attention. Several national governments are conducting control measures or making preliminary studies of their local problems. A joint study group of the Office International d'Hygiene Publique and the World Health Organization held a meeting on Bilharziasis in Cairo in October, 1949. Subsequently the World Health Organization appointed an Expert Committee on Bilharziasis, four members of which, Drs. Faust, McMullen, Oliver-González and Wright, are members of this Society. This Expert Committee held its first meeting in San Juan, Puerto Rico, in October, 1952, and I have had the use of its mimeographed report (WHO, 1952) and the individual contributions of its members in the preparation of this address. I have also drawn heavily upon the comprehensive and beautifully written paper by Faust (in press) on the Control of Schistosomiasis, delivered at the fiftieth anniversary celebration of the Institute of Tropical Medicine of Lisbon in 1952.

Problems in the control of schistosomiasis are related to the life cycles of the worms, the snail vectors, the customs and habits of the human host, and the nature of the environment in which all these elements come together.

The three species of schistosome worms in man have essentially the same life cycle, but are different enough to be considered three different diseases. *Schistosoma haematobium*, migrating as adult worms from the portal-mesenteric to the pelvic veins, lays its eggs largely in the venules of the urinary bladder so that they are extruded in the urine. *S. mansoni* and *S. japonicum*, remaining as a rule in the portal-mesenteric veins, lay their eggs mainly in the intestinal venules so that they are extruded in the feces. But these two species differ in that *S. japonicum* deposits many more eggs than *S. mansoni*, so that the disease is more often acute, and the ultimate damage, especially to the liver, is likely to be greater and the mortality higher. In the snail intermediate hosts there are also important differences. Those of *S. haematobium* and *S. mansoni* are hermaphroditic, do not have an operculum and therefore are entirely aquatic, whereas those

[1] The Eighteenth Annual Charles Franklin Craig Lecture, before the American Society of Tropical Medicine and Hygiene, November 12, 1953.

of *S. japonicum* are diecious; that is, have separate sexes, are operculate, and are amphibious, tending to crawl out of the water. They can survive for a long period in the dry state. In geographic distribution, *S. haematobium* is confined to Africa and adjacent areas. *S. mansoni*, endemic less extensively in Africa, was brought to the Western Hemisphere in African slaves and found suitable snail hosts so that it became endemic in South America and the West Indies. *S. japonicum* is confined to the Far East, with extensive endemic areas in Central and South China, several islands in the Philippines, and small areas in Japan, Formosa and the Celebes.

The common denominator in the epidemiology of all three types of schistosomiasis is that man becomes infected by exposure of the skin to fresh water containing cercariae which have emerged from snail intermediate hosts. The great variety of circumstances under which this exposure occurs produces the complicated picture which has to be considered in the control of the infection. Occupation (especially rice farming), bathing, fishing, washing clothes, performing religious ceremonies and participating in military operations, are probably the most frequent of these circumstances.

A more detailed review of the life cycle of the schistosomes will bring out the points at which the cycle might be broken in a program of prevention. When the infective larvae (cercariae) escape from the snail they must find and penetrate the skin of man within 48 hours in order to survive. They can penetrate the unbroken skin within a few minutes and must then gain access to the blood stream and work their way through the capillary barriers of the lungs and gastrointestinal tract to gain access to the mesenteric-portal venous system. Here they mature, mate, and *in copula* crawl back against the blood stream to the veins of the intestine or, beyond, to the urinary bladder where the female deposits the eggs in the small venules or capillaries. The embryo (miracidium) within the egg shell secretes a toxic substance which causes necrosis of the surrounding tissue, permitting the egg to escape into the lumen of the viscus and be excreted in the feces or urine. If the egg manages to enter fresh water, the egg shell swells and ruptures within a few hours, liberating the ciliated miracidium which must find and penetrate into a suitable snail host within 48 hours in order to survive. Within the snail one miracidium may give rise to thousands of cercariae.

Theoretically this seems to be a hazardous life cycle, but where the combination of conditions necessary for its completion exists, infection once introduced into a community can gradually spread to involve practically all of its inhabitants, and infections can increase in intensity so as to produce severe disability and high mortality. In the endemic areas there are wide variations in incidence and intensity of infection, often within short distances, due to slight differences in one or more of the factors related to endemicity. In some endemic areas of *S. haematobium* infection in Africa, as reported by Blair (1952), the infection usually causes little disability when measured by the ability of the population to carry on light activity. In such areas bloody urine is considered the normal state. In other localities, and especially in the endemic regions of

S. japonicum, heavy infections may lead to great disability and early death of a considerable proportion of the population.

There is good evidence that the endemic areas of all three species of schistosome have increased recently and will continue to increase. Movement of troops during military operations, improvement in transportation, migration of people for employment, and the expansion of irrigation systems have all been responsible for extension of the infection where suitable snail hosts are present. That further extension to new areas widely separated from present endemic areas is theoretically possible is illustrated by the temporary focus of *S. haematobium* that developed in Australia after World War I (Faust, 1949), and by the discovery that in the United States certain native snails are potential intermediate hosts for *S. mansoni* (Cram *et al.*, 1945), and *S. japonicum* (Berry and Rue, 1948).

The life cycle of the schistosomes appears to offer several vulnerable points at which theoretically it can be broken. The adult worms can be attacked by chemotherapy. Human excreta containing eggs can be prevented from entering fresh water, or this can be delayed until the miracidia within the eggs are dead. The snail intermediate host can be attacked by chemical and biological methods. Man can be instructed to avoid contact with potentially infested water, or his skin can be protected from cercariae. Each of these methods has received attention.

CHEMOTHERAPY

Chemotherapy on a mass scale was started in Egypt in 1919. Potassium antimony tartrate intravenously, and later antimony pyrocatechin sodium sulfonate (Fouadin) intramuscularly, were used in this campaign. By 1945 over 6,500,000 cases had been treated, with an average number of 9 injections per case in that year. The effect of this program (Shousha, 1949) was to decrease considerably the frequency of surgical complications of *S. haematobium* infection, but the incidence of infection was not materially affected. The difficulty of holding patients for a full course of treatment, because of the frequent toxic reactions, was largely responsible for failures to cure. Recent attempts to give more intensive treatment with sodium antimony tartrate have not been encouraging as a mass procedure. More hopeful has been the discovery of a non-antimonial compound, a complex thioxanthone derivative (Miracil-D, lucanthone, nilodin) which can be taken by mouth. The hydrochloride salt causes some unpleasant side reactions, such as abdominal pain, nausea, constipation and mental depression, but no serious toxicity. Courses of treatment of only three to five days have been sufficient to stop the discharge of eggs of *S. haematobium* in the urine, but recurrences have been common, probably because the female worms have been only temporarily disabled and resume normal egg laying in a few weeks. The careful recent work of Newsome (1951) shows how important it is to use accurate techniques of examination, even including rectal biopsy, to determine the effectiveness of chemotherapy. Preliminary observations by Newsome also indicated that Miracil-D salicylate was as effective as

the hydrochloride and less irritating to the gastrointestinal tract. Still more recently Newsome (1953) reported favorable results in baboons infected with *S. mansoni*, using a new Miracil derivative 27T51.

Miracil-D is apparently less effective against *S. mansoni* than against *S. haematobium*, and is ineffective against *S. japonicum* (Pesigan *et al.*, 1951). It is also very expensive at the present time.

If chemotherapy is ever to play an important part in the conquest of schistosomiasis, it will have to be applied on a mass scale. This will require a drug which can be administered easily, is nontoxic, will require only a short period of treatment, and is inexpensive. Chemotherapy will never be very effective in controlling *S. japonicum* because of the reservoirs of infection among domestic animals including dogs and pigs, and wild animals, especially rodents. However, chemotherapy has a place in the general campaign against schistosomiasis because it can at least reduce the number of eggs discharged in the excreta, and thus assist in lowering the incidence of infected snails. It can also reduce disability and deaths, which are important considerations in the control program. Therefore the search for the ideal chemotherapeutic agent should be prosecuted more vigorously than at present. In the past this search has been conducted mainly by empirical methods. More recently the metabolic studies of Bueding (1951) and his co-workers on *S. mansoni* have suggested a more rational approach. Such studies should be extended, in addition to the continuation of the research by empirical methods.

EXCRETA DISPOSAL

The control of excreta disposal in most of the endemic regions of schistosomiasis is practically impossible at the present time. In extensive rice-growing areas it is economically necessary to use human feces for fertilizer, and fresh feces are said to be best for the seed beds. In Moslem countries ablution before prayer is a required religious rite, and although it is supposed to be performed in clean water, such water may be heavily infested with cercariae resulting from earlier ablutions or other contamination. Kuntz (1952) even found man-made ablution pools in the Yemen containing infected intermediate hosts of *S. mansoni*. In rural villages the women wash their night-soil buckets in streams or canals, and also wash the family clothes in the same water. Where privies are provided for the "wasting" of human excreta, they are often placed directly over a stream as the simplest water-carriage system of disposal. Promiscuous defecation and urination are always likely to be performed directly into or close to a body of water if one is available.

It is known that storage of an undiluted mixture of feces and urine for three weeks at ordinary temperature will kill the miracidia within schistosome eggs. This would require storage tanks in regions where excreta are used for fertilizing rice fields. The tanks would have to be used in rotation, and records kept of dates when the last excreta were added. It would also require the intelligent cooperation of individual farmers who would understand the reason for storage. Addition of chemicals to storage tanks or to night soil buckets for the purpose

of killing miracidia would incur an impossible expense unless the chemical had fertilizing value in itself and was very cheap. Further search for such a chemical to be used in favorable situations would be worth while.

Religious customs can sometimes be adapted to act as a safeguard rather than as a hazard to health. With the modern development of sanitation in Moslem countries, education of religious leaders as to hazards and remedies could gain their cooperation in devising methods for avoiding contamination of water containing snail vectors as well as avoiding exposure to infested water. This would also require instruction of the population in the reasons for adopting safer techniques of performing religious ceremonies.

Where human excreta are not used for fertilizer but are wasted, a program of privy building adapted to the local situation, and instruction of the population to avoid promiscuous defecation and urination, should contribute to the prevention not only of schistosomiasis but also of many other intestinal infections.

In areas enjoying a higher economic level it may be possible to install septic tanks with water-carriage toilets and subsurface disposal, which should eliminate the possibility of snail infection. It has been found that even modern sewage treatment will not destroy the eggs or miracidia of *S. japonicum* (Newton *et al.*, 1948). But municipal sewerage systems emptying even raw excreta into streams are probably seldom the source of snail infections because of the high dilution and the absence of snails.

The problem of excreta disposal should be studied in each local situation to determine whether and how it can be incorporated into the general program. Its importance as a general health measure emphasizes the value of giving it serious consideration.

PROTECTION AGAINST INFECTION

Protection against infection requires either avoidance of entry into infested water or physical or chemical protection of the skin from attack by cercariae. Infested water cannot be detected by its appearance. Although polluted by excreta it is often clear and attractive. It is usually a still body of water, relatively small and shallow. If it is a large lake or a running stream, the dangerous places are the side pools where there is little movement of the water. In flooded rice fields the cercariae usually have been transported from a snail habitat in a stream or irrigation ditch.

It is obviously impractical to attempt to keep rice farmers who use the wet method of cultivation from entering the water of their fields, especially at the time of transplanting the young plants from the seed beds to the permanent fields, both of which are flooded for this procedure. It is also impractical to have them wear any kind of garment to protect their legs and arms. The application of an ointment containing a cercaricide, such as was developed during World War II by Wright and his colleagues (1948a), might be advocated, but it never could be guaranteed to protect, and would probably only be used in emergencies such as military operations.

Safe water for washing clothes can be provided in certain situations. In a

village where the women wash clothes in a stream containing infected snails, it might be possible to provide a community washing place supplied from a deep well. I believe this has been done in Puerto Rico. Chlorination of water with the dosage used to kill pathogenic bacteria will also kill cercariae.

Protective clothing for persons who must enter infested water is the other possible method of avoiding infection. Any kind of clothing to cover the limbs would be impractical for natives of endemic areas, either in their occupation, in performing religious rites or in bathing for sport or cleanliness. In military operations, protective clothing, including leather boots and trousers tucked inside the boots, would offer considerable mechanical protection (Hunter *et al.*, 1949). If impregnated with cercaricidal chemicals such as benzyl benzoate or dibutyl phthalate, better protection would be afforded (Wright *et al.*, 1948b). Protection of the hands and arms would be more difficult. These methods of prevention are adjuncts which might be used under certain circumstances but are insignificant in the vast problem involving the native population of endemic areas.

ERADICATION OF THE INTERMEDIATE HOST

Breaking the life cycle of the schistosomes by eradicating the snail intermediate hosts has the advantage that it can be conducted by governmental agents without the necessary cooperation of the people. It requires a thorough knowledge of the biology of the various species of snails involved, their habitats, susceptibility to attack, natural enemies, and resistance to adverse conditions.

Methods of attack on the snail have been divided by McMullen (1952) into biological and molluscicidal. The biological methods include changes in the physical environment, such as drainage, filling, flushing and removing vegetation; and encouragement of natural enemies of the snails which might include fish, aquatic fowl, aquatic insect larvae, worms, bacteria, fungi or viruses. Changes in the environment have received considerable attention in Egypt, by periodic drying of irrigation canals, removing vegetation, collecting snails on palm leaves, etc., but these methods have produced only temporary reduction in the snail population. Even the aquatic, nonoperculate hosts of *S. mansoni* and *S. haematobium* can survive in the dried mud of a canal bottom for several weeks by sealing the shell opening with a mucoid plug. Changes in environment made by man in developing agriculture, especially by irrigation, have been responsible for the extension of favorable habitats for the snails. If a method of rice cultivation could be developed which would make flooding of the fields unnecessary at a time when the farmers have to enter the fields, the disease might no longer be primarily a rice farmer's disease. This is a good research problem for agronomists.

The fact that snail colonies are known to have disappeared, at least temporarily, in Puerto Rico (Oliver-González 1953) without any obvious change in the physical environment, indicates that biological control by some as yet unknown agent may be possible in certain situations. This phenomenon is now being studied in Puerto Rico.

The eradication of snails by chemical molluscicides has been attempted for many years and is now receiving increased attention. The Japanese have used calcium cyanamid, a good fertilizer, but it is expensive and is useful only against amphibious snails. Following the discovery by Chandler (1920) that copper sulfate was an effective molluscicide it was used extensively in Egypt and has been employed also in Brazil and Venezuela. It is cheap and nontoxic to higher animals, but it combines too readily with organic matter and it does not kill the eggs of snails. It is effective against amphibious snails only when they are in the water.

With the infection of many American troops by *S. japonicum* in the Philippines during World War II a search was started for better molluscicides against the amphibious *Oncomelania* snails. Beginning with the work of McMullen and Graham (1947) thousands of chemical compounds have been screened in laboratory tests against both amphibious and aquatic snails and many of those showing promising activity have been field-tested (McMullen, 1952a; Nolan *et al.*, 1953). The most effective and practical compounds discovered thus far are two phenolic derivatives, dinitro-ortho-cyclohexylphenol, and sodium pentachlorophenate. They are about equally effective, and come near to fulfilling the qualifications of an ideal molluscicide as stated by McMullen (1952b) as follows: "The chemical should be toxic to snails and eggs in all stages at a low concentration; it should not be dangerously toxic to man, domestic animals or crops; it should not be easily inactivated by physical or chemical means; it should be cheap; and it should be easy to handle." These compounds are not yet cheap, but should become so with mass production. The dinitro compound can be applied either as a dust or in aqueous solution, but sodium pentachlorophenate dust is very irritating to the nasal mucosa and it can therefore be applied only in solution. Extensive field tests have been conducted with both compounds in Japan (McMullen *et al.*, 1951; Hunter *et al.*, 1952), and a five year plan for control of the snail with pentachlorophenate in the entire endemic area of Kyushu has been advocated (Hunter *et al.*, 1952). In Puerto Rico, Berry, Nolan, and Oliver-González (1950) found the pentachlorophenate effective against *Australorbis glabratus* in a running stream. In Egypt, Kuntz and Wells (1951) found the dinitro compound much more effective than copper sulfate against *Biomphalaria boisyi* and *Bulinus contortus* in still water, and demonstrated the persistence of its molluscicidal action as long as four weeks after application to water. They emphasized the economy of conducting snail control in the warmest months because molluscicides have more lethal action in warm water. They advocated continuing the use of copper sulfate in running water for economic reasons. In the Philippines the preliminary work of McMullen and Graham (1947) with the dinitro compound was extended by Pesigan and Masilungan (1950) with fair results, but a recent study of the habitat of *Oncomelania quadrasi* by a WHO team (McMullen *et al.*, 1953) discovered that the snail is present even in stream sources in virgin forest, and recommended that the biology and ecology of the snail be studied further before attempting its control with molluscicides.

Alves and Blair (1953) have recently reported an interesting combined pro-

gram for controlling both malaria and schistosomiasis in Southern Rhodesia. The same personnel and equipment are used in different seasons for house spraying to kill mosquitoes and for spraying streams with a solution of copper sulfate to kill snails. The effect of this program on the prevalence of schistosomiasis will not be known for several years. Meanwhile the government intends to extend the operations gradually until all economically important areas are covered.

The subject of molluscicides may be summarized by saying that although they seem to be the simplest approach to control of schistosomiasis, they have not so far completely eradicated the snails in any experimental field control program. The newer effective compounds are at present too expensive to be used in any large endemic region, and there are many endemic areas where they would be ineffective. The time is ripe, however, for vigorous trial of this method of control in small endemic areas such as those in Japan, Puerto Rico and the Dominican Republic.

CONCLUSION

We are now in a stage in the conquest of schistosomiasis comparable to the stage in malaria control when we had only quinine for treatment, oil, paris green and ditching for larva control, and fly swatters and screening for adult mosquitoes. Perhaps an ideal drug for treatment and a cheap and more highly effective molluscicide will be discovered to simplify the attack on schistosomiasis. Such efforts must be vigorously prosecuted and generously supported. Meanwhile it is important to continue basic studies of both the metabolism of the worms and the biology and ecology of the intermediate hosts so as to approach the problem more intelligently. There are few endemic areas where one method alone can accomplish control. Each area must be studied and a combination of methods adopted which will meet the local situation. As McMullen (1952b) has aptly stated, "The successful attack on this disease will be accomplished by a team; the engineer, the malacologist, the parasitologist, the sanitarian, the physician, and the chemist; widely divergent in training, speaking in the beginning different technical languages but with a common purpose in mind." I believe also that general education of people in hygiene, and specific education concerning the disease are important adjuncts to medical and public health measures. An intelligent population can be an important element in its own protection against disease. With concerted efforts on an international scale, research and administrative programs should bring progress in the conquest of schistosomiasis in progressively larger portions of its endemic regions.

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THE DURATION IN THE HUMAN HOST OF INFECTIONS WITH A PANAMA STRAIN OF *PLASMODIUM FALCIPARUM*

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The long duration of *Plasmodium falciparum* infections in the human host has been reported by Eyles and Young (1951). Using a South Carolina strain of *P. falciparum*, it was found that infections which were treated inadequately or not at all persisted for an average of 222 days. Three of the infections persisted for more than one year, the longest being 480 days. The present observations are an extension of this work to another strain of *P. falciparum*, one originating in Panama. It seemed desirable to determine if this strain originating in a tropical area differed materially from the temperate zone strain.

MATERIALS AND METHODS

The strain of *P. falciparum* used in this study was recovered in 1948 from a mestiza of El Limon, Panama, and is designated in this laboratory as P.F.-6. The patients studied were neurosyphilitic Negroes, inoculated either by sporozoites or by the intravenous injection of infected, citrated whole blood. Blood smears were started immediately in blood-inoculated cases and after five or six days in mosquito-inoculated cases. Daily blood smears were taken, with the exception of some Sundays and holidays, until at least one month following the disappearance of parasites, and in many cases were continued for a minimum examination period of six months following the last observed parasites. In other cases the one month negative daily blood smears were followed by a period of twice-weekly smears until at least six months had elapsed from the time parasites were last seen. In the event renewed parasite activity was observed, daily smears were reinitiated and the same procedure for negative periods followed. A few cases were in variance with this protocol, and these variations will be pointed out when the cases are discussed.

Daily blood smears were made by the Earle-Perez (1932) thick film method and 0.1 cmm. of blood was examined except when high parasitemias made a smaller volume sufficient for accurate determination of parasite densities. Densities of asexual parasites, and female and male gametocytes were routinely determined. The twice-weekly blood films were usually ordinary thick-thin preparations, but an amount of blood approximating 0.1 cmm., as determined by numbers of white blood cells, was examined in each of these. The blood films were stained with Giemsa and precautions were taken by isolation of probable negative films to prevent possible transfer of parasites from slide to slide during staining.

Patient temperature readings were initiated at the same time as the blood

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smears and were made at four-hour intervals during the clinical stages of the infection. When elevations were noted, readings were made hourly, or more often, during the febrile period. When the patient showed an unfavorable physical reaction to the infection, or when the parasitemias became dangerously high, the infection was either terminated or, more usually, reduced to manageable levels with small amounts of antimalarial drugs. Usually the latter was accomplished with five to ten grains of quinine, 50 to 100 mg. of Paludrine, or some combination of the two drugs. These treatments were repeated when indicated.

It should be noted that the methods used were identical with those of Eyles and Young (1951) in their study of the duration of the Santee-Cooper strain of *P. falciparum*, and the results therefore are directly comparable.

OBSERVATIONS

A total of 96 patients were inoculated during the study, but in only 39 was it possible to make sufficient observations for inclusion in the results. In 23 of these cases observations were continued throughout the duration of the infections and for sufficient periods following the last parasites. All 39 were followed through the long primary period of continuous parasitemia.

The general pattern of the infection in the Panama strain of *P. falciparum* was very similar to that of the Santee-Cooper strain described by Eyles and Young (1951). Among the significant differences was the usually greater severity of the primary clinical period in the Panama strain. As in the Santee-Cooper strain, periods of renewed clinical activity occasionally occurred and a long period of asymptomatic continuous parasitemia followed. Following this, intermittent parasitemias were seen for a period of varying duration. These features are discussed more thoroughly in the following paragraphs.

TABLE 1

Characteristics of the clinical attack in a Panama strain of P. falciparum

	PANAMA STRAIN			SANTEE-COOPER STRAIN*
	Sporozoite	Blood	Total	Total
Number of patients	24	13	37	38
Number where infection was partially suppressed	12	6	18	8
Mean length of initial clinical episode—days, with S.E.	13.1 ± 1.2	10.7 ± 1.4	12.3	7.0
Mean no. of clinical episodes, with S.E.	3.3 ± 0.36	2.4 ± 0.27	2.9	1.5
Mean total hours of fever over 101°F. oral, with S.E.	124.8 ± 12.4	77.5 ± 8.9	108.2	60.5
Median maximum parasite count (per cmm.)	49,121	75,600	70,560	74,000
Mean maximum parasite count	73,740.9	82,483.8	76,812.8	?

* Eyles and Young, 1951.

The clinical attack. The clinical attack in the Panama strain is quite severe. Table 1 summarizes the characteristics of the clinical attack. The corresponding data (totals only) for the Santee-Cooper strain (Eyles and Young, 1951) are included for comparison.

It will be noted that in about half of the Panama strain cases partial suppression with antimalarial drugs was necessary. In the total number of cases inoculated the ratio would be considerably higher since in many cases it was necessary to terminate the infection during the primary clinical attack, or to suppress it to such an extent as to make the observations unusable for the purpose of this study.

From Table 1 it can be seen that the Panama strain is more virulent than the Santee-Cooper strain. This is especially noticeable in the mean length of the clinical period, the number of clinical episodes and the total hours of fever. The maximum parasite density is nearly the same, but probably reflects the effect of frequent use of drugs for the temporary suppression of parasite levels. These drugs have a somewhat greater effect on the parasite densities than they do on the clinical symptoms when used for this partial suppression. Actually, suppressives are usually given at a time when it is known that they will prevent extremely high parasitemias during the initial wave of asexual parasites. Hence, the use of maximum parasite level as a criterion for severity of clinical attack may be somewhat misleading.

There is an apparent difference in severity of the clinical attack between sporozoite and blood inoculated cases. Significantly greater in the sporozoite inoculated cases were the mean number of clinical episodes ($P = 0.045$) and total hours of fever ($P = 0.002$). The length of the initial clinical episode did not differ significantly ($P = 0.190$) in blood and sporozoite induced cases.

Continuous, remittent parasitemias. This period, including and following the clinical episodes, is characterized by successive waves of asexual parasites and gametocytes. The mean length of this period (Table 2) was 115.7 ± 7.5 days, with a range of from 36 to 220 days, and with little difference between the blood and sporozoite induced cases. Likewise, the length of this period did not differ significantly from the corresponding period in the Santee-Cooper strain.

Terminal intermittent parasitemias and over-all duration of the infection. A total of 23 cases were followed through the period of terminal intermittent parasitemia. The mean duration of this period was 168.3 ± 19.0 days. This duration was significantly longer ($P = 0.016$) than the mean 100 day period observed by Eyles and Young for the Santee-Cooper strains (Table 2).

With a few exceptions these 23 cases were followed until no parasites had been seen for six months. It was necessary to terminate observations on four before completion of the total observation period, but these were infections of such long duration (253, 353, 365, and 395 days) that they are included in the results. It is judged probable that these infections had completed their full course. Two other infections have not as yet completed the six-month observation period, but again are included because of the long duration of the infection (362 and 503 days). The mean total duration of the infection was 279.4 ± 19.9 days (Table 2),

TABLE 2

Duration of parasitemias in a Panama strain of P. falciparum

	PANAMA STRAIN			SANTEE-COOPER STRAIN*
	Sporozoite	Blood	Total	Total
Total duration				
No. of cases	17	6	23	22
Mean days with S.E.	291.1 ± 22.6	246.5 ± 42.1	279.4 ± 19.9	222 ± 25
Standard deviation	93.1	103.2	95.5	117
Extremes, days	114–503	130–365	114–503	32–480
Duration of initial period				
No. of cases	27	12	39	38
Mean days with S.E.	118.7 ± 8.8	109.0 ± 14.8	115.7 ± 7.5	121 ± 9
Standard deviation	45.6	51.2	47.1	58
Extremes, days	46–220	36–196	36–220	32–224
Duration of terminal intermittant				
No. of cases	17	6	23	22
Mean days, with S.E.	172.5 ± 23.8	156.5 ± 30.1	168.3 ± 19.0	100 ± 21
Standard deviation	98.2	73.6	91.1	85
Extremes, days	34–394	82–257	34–394	0–283

* Eyles and Young, 1951.

or about 57 days longer than that seen in the Santee-Cooper strain. Although this difference is not statistically significant in the usual sense, the probability is strong ($P = 0.075$) that a real strain difference is represented by these observations. Variation in duration was considerable, with extremes of 114 and 503 days. There was little difference between sporozoite and blood-induced infections, where total duration was concerned.

DISCUSSION

The observations reported confirm the findings of Eyles and Young as to the long persistence of *P. falciparum* infections. Actually, the evidence indicates that the Panama strain persists somewhat longer on the average than the indigenous strain previously studied; thus, the present study establishes more securely that this long duration is a characteristic of the species.

In the case of the temperate zone strain studied by Eyles and Young, the long duration of infection seemed to offer a logical explanation of the carrying over of the parasite through the winter until the next transmission season. Although this consideration may be of lesser importance in some tropical areas, dry seasons limit transmission in others and make a mechanism of conservation necessary for the survival of the parasite. The relationship of the long-persistent infections to potential mosquito transmission has been studied experimentally in some of the patients. The data thus obtained will be the subject of a forthcoming report.

Previous reports (Eyles and Young, 1950; Jeffery *et al.*, 1950) have shown that the Panama strain of *P. falciparum* is more infective to *Anopheles albimanus* than to *A. quadrimaculatus*; the reverse is true with the Santee-Cooper strain. This constitutes a measurable strain difference, to which can be added others brought out in the present report.

In the first place, the Panama strain appears to be more virulent than the Santee-Cooper strain as indicated by the mean length of the initial clinical episode, the mean number of clinical episodes, and the mean total hours of fever. Each of these figures was about double the corresponding figure for the Santee-Cooper strain. The fact that the mean maximum parasite levels were approximately the same in the two strains is probably due to the fact that treatment was used to control the parasite level in a larger proportion of the patients with the Panama strain, and it was likewise necessary to terminate the malaria radically in a larger proportion of the total number of patients inoculated for the study.

A third measurable difference between the two strains is the longer duration of terminal, intermittent parasitemia and the probable longer total duration of infection in the Panama strain. As was pointed out previously, the difference in total duration is not statistically significant if the usual criterion of significance is followed, but on the basis of the evidence at hand it would appear more likely that the Panama strain endures longer than that the persistence is of the same magnitude.

It has also been observed in this laboratory that little cross-immunity exists between the two strains. Differences in morphology have been noted. The gametocytes of the Santee-Cooper strain are generally of a typical sausage-shape, while those of the Panama strain are quite variable in shape and appear to be smaller in size.

SUMMARY AND CONCLUSIONS

In a Panama strain of *Plasmodium falciparum* 23 inadequately treated infections persisted for an average of 279.5 ± 19.9 days, with a range in duration from 114 to 503 days. Four cases persisted for more than a year. The initial period of continuous parasitemias in 37 cases persisted for an average of 115.7 ± 7.5 days. This was followed in 23 cases by a period of terminal intermittent parasitemias with a mean duration of 168 ± 19.0 days. There was little difference between blood and sporozoite induced infections where duration of the infection was concerned.

The results reported extend to a tropical strain the findings of Eyles and Young (1951) on the duration of a temperate zone strain of *P. falciparum*. The application of these findings to certain field conditions is discussed.

The Panama strain of *falciparum* apparently is somewhat more virulent than the United States strain. Although the maximum parasite densities were similar in the two strains, the Panama strain was nearly double the U. S. strain in the length of initial clinical episode, number of clinical episodes and total hours of fever. Other criteria suggestive of strain difference are the significantly longer

duration of terminal intermittent parasitemias in the Panama strain, the absence of cross-immunity between the two strains, and differences in gametocyte morphology.

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ANTIMALARIAL ACTIVITY OF DIHYDROTRIAZINES[1]

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It has been reported by Carrington *et al.* (1951) that 2,4-diamino-1-(*p*-chlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine and 2,4-diamino-1-(3,4-dichlorophenyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine are respectfully 10 and 100 times more active than chlorguanide against *Plasmodium gallinaceum* in chickens. Schmidt *et al.* (1952), on the other hand, found the former compound to be 2 to 4 times less active than chlorguanide against *P. cynomolgi* in monkeys, whereas the latter was of the same order of activity as chlorguanide. The interest of these investigators in the antimalarial activity of triazines stemmed from previous studies on the metabolic fate of chlorguanide, in which it was demonstrated that this compound *per se* was inactive *in vitro* against *P. gallinaceum* and *P. cynomolgi* (Hawking, 1947; Geiman, (1948); Hawking and Perry (1948)). The triazines mentioned above were considered to be the "active" metabolites of chlorguanide and dichlorguanide.

In the present paper, the effects of various triazines against *P. lophurae* in ducks and *P. berghei* in mice are described, and compared with the activity of several other antimalarials. Although the activity of the two triazines tested by Schmidt *et al.* (1952) against *P. cynomolgi* was much less than against *P. gallinaceum*, this group might possess potentialities for the treatment of human malaria.

METHODS

In the early part of these investigations, the routine screening of compounds for antimalarial activity was carried out in ducks infected with *P. lophurae*, and the first group of triazines received from the Children's Cancer Research Foundation was tested only against *P. lophurae*. The KBG-173 strain of *P. berghei* was then furnished us through the kindness of the National Institutes of Health, Bethesda, Maryland. Comparative evaluations of antimalarial activity of subsequent members of the triazine series were performed in mice infected with *P. berghei*, due to the several advantages provided by this mammalian parasite in a small laboratory host.

P. lophurae. Infections with *P. lophurae* were produced in 2-week-old white Pekin ducklings by injecting intravenously 0.5 cc. of citrated blood from a heavily parasitized donor bird. Parasite counts were made on the 5th day from treated and control birds. The dosage of the compounds tested which reduced the parasitemia to approximately 10 per cent of that in control birds (ED_{90})

[1]The dihydrotriazines discussed herein were prepared in the laboratories of the Children's Cancer Research Foundation, Boston, Massachusetts, and were submitted to us by Dr. Sidney Farber for evaluation of activity against various types of animal parasites. We are indebted to Dr. Farber and his colleagues for supplying these compounds.

was chosen as the minimum effective dose. Compounds were administered orally by rubber catheter tube, twice daily for 3½ days. One dose was given shortly after inoculation with parasites, with two daily doses thereafter for 3 days.

P. berghei. Mature white mice were injected intraperitoneally with 0.1 cc. of blood (1:100 dilution in saline) from donor mice with 40 to 50 per cent of the red cells parasitized on the 5th to 7th days after inoculation. Parasite counts were made on the 6th or 7th day from treated and control mice. The calculated dosage (expressed in terms of the base) of the compounds which reduced parasitemia to approximately 10 per cent of that in control mice (ED_{90}) was chosen as the minimum effective dose. Treated mice were then held for a period of 30 days, and the calculated dosage of the active compounds which allowed survival of 50 per cent or more mice for this period was chosen as the minimum "curative" dose (CD_{50}). All nontreated control mice died within this period, generally before the 14th day. The term "curative" is used in a relative sense only, since it was not established for certain that absolute eradication of the parasites had been accomplished in any case.

Preliminary to testing triazines against *P. berghei*, considerable time was

TABLE 1

Comparison of quinine, chloroquine and sulfadiazine against P. berghei *in mice*

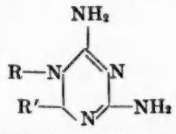
COMPOUND	NO. MICE USED	ORAL DOSAGE* (twice daily)		MEAN PARASITEMIA % OF CONTROLS 7TH DAY	% SURVIVALS		ED_{90} mg./kg.	CD_{50} mg./kg.
		mg./kg.	No. days treated		30 days	60 days		
Quinine	20	80	3	3.9	30	0	60	>80
	15	60	3	10.4	0	0		
	15	40	3	65.0	0	0		
	20	80	6	0.0	80	70	35	70
	20	60	6	0.4	45	35		
	20	40	6	7.3	10	5		
	20	30	6	15.9	0	0		
Chloroquine	20	13	3	0.0	60	60	<6	11
	20	10	3	0.0	50	40		
	20	6	3	0.0	25	20		
	20	6	6	0.0	70	65	2.5	5
	20	3	6	0.2	45	30		
	20	2	6	32.1	0	0		
Sulfadiazine	20	14	3	0.0	70	65	<5	9
	20	9	3	0.0	65	50		
	20	5	3	0.15	35	35		
	10	5	6	0.0	50	50	1.5	5
	10	2	6	1.3	20	10		
	10	1	6	13.0	0	0		

* All doses expressed in terms of the base.

spent in evaluating the activity of other known antimalarials against this parasite. In this respect, representative data are given in Table 1 regarding quinine, chloroquine and sulfadiazine. Quinine is not highly effective against *P. berghei*, as has been demonstrated previously by Goodwin (1949), Hill (1950), and Thurston (1950). When treatment was given for six days rather than three days, the ED_{90} was halved, but from the standpoint of total dosage this difference

TABLE 2

Comparison of dihydrotriazines against P. berghei *in mice*



REF. NO.	R =	R′ =	ED_{90} (mg./kg. BID ORAL, 6 DAYS)*	CD_{10} (mg./kg. BID ORAL, 6 DAYS)*	QUININE EQUIVALENT		CHLORGUANIDE EQUIVALENT
					ED_{90}	CD_{10}	
D31	phenyl	*n*-propyl	14	>14	3	<5	<1
D45	*p*-chlorophenyl	methyl	3	11	13	7	1
D60	*p*-chlorophenyl	ethyl	2	11	19	7	2
D48	*p*-chlorophenyl	*n*-propyl	2	11	19	7	2
D59	*p*-chlorophenyl	*iso*-propyl	>45	—	<1	—	<0.1
D20	*p*-chlorophenyl	dimethyl	2	11	19	7	2
D43	*p*-chlorophenyl	methyl, ethyl	5	>14	7	<5	<1
D37	*p*-chlorophenyl	phenyl	11	>14	3	<5	<1
D47	*p*-chlorophenyl	*o*-chlorophenyl	>14	—	<3	—	<1
D64	*p*-chlorophenyl	tetra-methylene	>14	—	<3	—	<1
D40	*p*-chlorophenyl	penta-methylene	>45	—	<1	—	<0.1
D65	*p*-bromophenyl	dimethyl	2	>14	19	<5	2
D67	3,4-dichlorophenyl	methyl	0.7	<2	50	>40	5
D63	3,4-dichlorophenyl	ethyl	3	5	13	14	1
D76	3,4-dichlorophenyl	*n*-propyl	7	>14	5	<5	<1
D62	3,4-dichlorophenyl	*iso*-propyl	>45	—	<1	—	<0.1
D54	3,4-dichlorophenyl	dimethyl	0.4	5	88	14	9
D75	3,4-dichlorophenyl	methyl, ethyl	4	>14	9	<5	<1
D55	3,4-dichlorophenyl	phenyl	11	>14	3	<5	<1
D53	2,4-dichlorophenyl	dimethyl	>45	—	<1	—	<0.1
D57	2,4-dichlorophenyl	phenyl	>45	—	<1	—	<0.1
D56	2,5-dichlorophenyl	dimethyl	>45	—	<1	—	<0.1
D69	*m*-chlorophenyl	dimethyl	0.7	11	50	7	5
D68	*o*-chlorophenyl	dimethyl	>45	—	<1	—	<0.1
D38	*o*-tolyl	dimethyl	>45	—	<1	—	<0.1
D72	*o*-tolyl	tetra-methylene	>45	—	<1	—	<0.1
D49	*p*-anisyl	*n*-propyl	>45	—	<1	—	<0.1
D27	*p*-carboxamidophenyl	dimethyl	>45	—	<1	—	<0.1
	Quinine		35	70	1	1	0.1
	Chlorguanide		3.5	—†	10	—†	1

* All doses expressed in terms of the base.

† CD_{10} greater than tolerated doses.

is not significant. Chloroquine and sulfadiazine were considerably more active than quinine, but the difference between 3-day and 6-day treatment periods was likewise not significant when computed on the basis of total dosage administered. It is of interest that although the relative activities of quinine and chloroquine obtained against *P. berghei* were similar to those reported by Hill (1950) and Thurston (1950), sulfadiazine was found to be much less effective than the figures given by these authors. Hill (1950) for example, gives the quinine equivalent of sulfadiazine as 2,000, whereas Thurston (1950) reports it to be 727. From our data (Table 1), the quinine equivalent of sulfadiazine is 23. (The sodium salt of sulfadiazine was used for treatment, and the ED_{90} was calculated in terms of the base.) This enormous variation is difficult to explain, even though allowances are made for variations in the minimum effective dose when computed by different methods. Moreover, our quinine equivalents relative to chloroquine and chlorguanide are very close to those reported by Hill (1950) and Thurston (1950). It may be that through repeated rapid passage our strain of *P. berghei* has become more virulent, and that this increase in virulence is accompanied by a reduced susceptibility to sulfadiazine. In this respect, the mean percentage of parasitized erythrocytes in our untreated control mice ranged from 35 to 50 on the 6th or 7th day after inoculation. This is similar to figures given by Mercado and Coatney (1951). Hill (1950) gives the mean percentage of parasitized erythrocytes on the 8th day to be 3.7, and Thurston's (1950) figure is 16.3 on the 5th or 6th day.

In testing the triazines listed in Table 2 against *P. berghei*, the 6-day treatment period was followed, even though experience with other antimalarials demonstrated that little advantage would be likely over the 3-day treatment period when comparisons were made on the basis of total dosage administered. It was felt, however, that the maximum treatment period possible before the peak of parasitemia was reached in untreated controls might reveal differences in degree of activity that could be missed otherwise, particularly with regard to the CD_{50}. Both quinine and chlorguanide equivalents for the ED_{90} of the triazines tested are given in Table 2. With the 6-day treatment period, however, it was not possible to compute CD_{50} chlorguanide equivalents, since the dose of chlorguanide required to arrive at this figure was greater than the tolerated dose in mice. With further regard to the CD_{50}, a holding period of 60 days did not give results significantly different from the 30-day period (Table 1).

RESULTS

P. lophurae. Of 6 triazines tested against *P. lophurae* infections in ducks, only one gave a quinine or chlorguanide equivalent greater than 1. This was 4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (D20) which was twice as active as quinine or chlorguanide (when expressed in terms of the base, the ED_{90} of the latter compounds was identical against *P. lophurae*). Two other triazines were active to a lesser degree. These were: 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine (D23, Q = 0.6) and 4,6-diamino-1,2-dihydro-1,2-diphenyl-*s*-triazine (D34, Q = 0.1). No activity was obtained

at oral doses of 200 mg. per kg. with 4,6-diamino-1-(*p*-carbethoxyphenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (DX19), 4-amino-6-anilino-1,2-dihydro-2 phenyl-*s*-triazine (DX34) or 4-amino-6-(*p*-chloroanilino)-1,2-dihydro-2,2-dimethyl-*s*-triazine (DX20). Although a sufficient number of triazines were not tested against *P. lophurae* to arrive at conclusions regarding the most favorable substitutions for further syntheses, the degree of activity of compound D20, as compared with several other antimalarials, was such that further studies of the group appeared to be warranted. It should be emphasized that *P. lophurae* is frequently much less sensitive to antimalarials than is *P. gallinaceum*. For example, Daraprim has been given a chlorguanide equivalent of 60 when tested against *P. gallinaceum* (Falco *et al.* 1951), whereas we have found it to be only 4 times more effective than chlorguanide against *P. lophurae*. The minimum effective doses (ED_{90}) of quinine, quinacrine and chlorguanide against *P. lophurae* are practically identical when expressed in terms of the base, but the latter two compounds are several times more effective than quinine against *P. gallinaceum*.

P. berghei. It will be noted (Table 2) that, with 3 exceptions, all 1-phenyl; 1-(*p*-chlorophenyl); 1-(*p*-bromophenyl); 1-(3,4-dichlorophenyl); and 1-(*m*-chlorophenyl)dihydrotriazines tested were active to some degree at least against *P. berghei* in mice. The exceptions were 2-(iso-propyl), and 2-pentamethylene derivatives (D59, D62, D40). Seven triazines gave quinine equivalents of 19 or greater and chlorguanide equivalents of from 2 to 9, when the ED_{90} was used for comparative evaluations (D60, D48, D20, D65, D67, D5A, D69). On the other hand, only three triazines (D63, D54, D67) gave quinine equivalents of 13 or greater when the CD_{50} was used for comparative evaluations. In this connection, the most effective triazines were 1-(3,4-dichlorophenyl) compounds.

In Table 3, data are given regarding the comparative effectiveness against *P. berghei* of several different types of antimalarials as compared with two of the most active triazines which we have tested thus far. Of particular interest

TABLE 3

Comparison of activity of two dihydrotriazines (D67, D54) with several other antimalarials against P. berghei *in mice*

COMPOUND	ED_{90} (mg./kg. BID ORAL, 6 DAYS)*	CD_{50} (mg./kg. BID ORAL, 6 DAYS)*	QUININE EQUIVALENT		CHLORGUANIDE EQUIVALENT ED_{90}
			ED_{90}	CD_{50}	
Quinine	35	70	1	1	0.01
Primaquinine	3.5	7	10	10	1
Chlorguanide	3.5	—†	10	—†	1
Chloroquine	2.5	5	14	14	1.4
Sulfadiazine	1.5	5	23	14	2.3
D67	0.7	<2	50	>40	5
D54	0.4	5	88	14	9
Daraprim	0.1	0.5	350	140	35

* All doses expressed in terms of the base.

† CD_{50} greater than the maximum tolerated dose.

are similarities and differences in the quinine equivalents when computed on the basis of the ED_{90} as compared with the CD_{50}. With primaquine, chloroquine and D67, quinine equivalents computed either from the ED_{90} or the CD_{50} are similar in each case, whereas the equivalents of sulfadiazine, D54 and Daraprim are considerably less when determined from the CD_{50} than when the ED_{90} is used.

DISCUSSION

Although *P. berghei* has not been employed as widely for evaluating antimalarials experimentally as have certain of the avian and simian plasmodia, data obtained by several investigators thus far have demonstrated its usefulness in this connection (Goodwin 1949; Schneider *et al.*, 1949; Schneider and Montezin 1950; Hill, 1950; Thurston, 1950). Falco *et al.* (1951) and Rollo (1952) have more recently found it helpful in the comparative evaluation of activity of 2,4-diaminopyrimidines. The fact that *P. berghei* is either more or less sensitive than the human plasmodia to certain types of antimalarials does not detract from its value for the measurement of activity within a group of compounds of similar structure. The vast amount of data which has accumulated during the years following our entry into World War II indicates clearly that the best that can be expected of any host-parasite relationship for assessing antimalarial activity in the laboratory is to suggest the potentialities of the most active members of a series of similar chemical compounds for use in man. The carry-over of results from experimental animals to man can unfortunately seldom be predicted with any degree of accuracy, regardless of the variety of experimental tests to which a compound is submitted. The use of *P. berghei* in mice for assessing antimalarial activity provides a small, easily manageable, mammalian host-parasite relationship, with the added advantage that relatively small amounts of compound are required for assay. It is susceptible to the action of all known antimalarials which have been tested and reported thus far, and there is little reason to suspect that it will prove any less efficient than avian or simian plasmodia for the preliminary steps in evaluating activity against the erythrocytic stages at least.

In the case of the dihydrotriazines described herein, several are more active than quinine, primaquine, chlorguanide and chloroquine against *P. berghei*. The degree of activity of several members of the triazine series tested thus far is sufficient to justify further investigation of similar compounds, and this is in progress. The number tested is as yet too small to allow conclusions regarding types of derivatives most likely to have the most potent activity, although 1-(3,4-dichlorophenyl) compounds appear to possess the best potential in this respect.

SUMMARY

Data are given regarding the effects of several dihydrotriazines against *P. lophurae* in ducks or *P. berghei* in mice. A number of the compounds tested were more active than quinine, primaquine, chlorguanide and chloroquine

against *P. berghei*. Among the most active were 4,6-diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2-methyl-*s*-triazine and 4,6-diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine.

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FACTORS AFFECTING PASSIVE IMMUNITY IN BLOOD-INDUCED *LOPHURAE* MALARIA OF THE CHICKEN[1,2]

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Passive immunity against *Plasmodium lophurae* was first demonstrated by Taliaferro and Taliaferro (1940). These workers were able partially to protect chickens against infection with injections of serum from chickens which had been infected by inoculation with parasitized chicken erythrocytes, had recovered from the infection, and had subsequently been superinfected by inoculation with parasitized chicken erythrocytes.

But when a chicken receives injections of parasitized chicken erythrocytes, it receives more than just parasites. The erythrocytes in which the parasites are residing and the accompanying noninfected erythrocytes may also stimulate antibody production by the recipient (Todd, 1930). The possibility must be considered that the protective action of sera from chickens infected and superinfected by inoculation with parasitized chicken erythrocytes may be partially or wholly due to anti-erythrocytic antibodies.

Eaton and Coggeshall (1939) reported that the *in vitro* antiplasmodial activity of sera from rabbits immunized with parasitized (*P. knowlesi*) monkey erythrocytes could be absorbed out with normal monkey erythrocytes. Rabbit serum obtained after immunization with nonparasitized monkey erythrocytes was effective against *P. knowlesi in vitro*, but normal rabbit serum was not. Zuckerman (1945), working with *P. gallinaceum* and with *P. lophurae*, observed agglutination and phagocytosis of both parasitized and nonparasitized chicken erythrocytes in the presence of sera from chickens superinfected a number of times with parasitized chicken erythrocytes. The opsonin could be absorbed from *gallinaceum*-immune serum more effectively with nonparasitized cells than with parasitized cells. Coffin (1951) was able to absorb *in vitro* protective activity from hyperimmune duck sera (from ducks infected and superinfected with *P. lophurae* by inoculation with parasitized duck erythrocytes) with residues from lysed nonparasitized duck erythroyctes. Hyperimmune duck sera strongly agglutinated nonparasitized duck erythrocytes, while sera from noninfected ducks agglutinated such erythrocytes slightly or not at all. Coggeshall (1940) reported that a human serum containing very high-titered agglutinins for normal monkey erythrocytes was very effective in protection of monkeys against *P. knowlesi* infections induced with parasitized monkey erythrocytes.

[1] Most of this material is drawn from Iowa State College doctoral thesis number 1392, two copies of which are on file in the Iowa State College Library.

[2] This study was sponsored by the Army Medical Service Graduate School, and was supported in part by the Medical Research and Development Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

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Anti-erythrocytic isoantibodies induced in chickens by inoculation with chicken erythrocytes can often be demonstrated by the agglutination reaction (Todd, 1930). The *in vitro* reactions, as well as the *in vivo* antimalarial activity, of plasmas from immunized and nonimmunized chickens were studied. Such *in vitro* studies might be expected to add to our knowledge of the *in vivo* reaction.

In a brief preliminary report (Schwink and Becker, 1951) on these agglutination investigations, it was reported that freezing of isoagglutinating chicken plasmas markedly enhanced their agglutinating ability. The effect of supplements of other plasmas upon the agglutination depended largely on whether the agglutinating plasma had been previously frozen and thawed. With previously frozen agglutinating plasma, the effect was generally inhibition of the agglutination. With unfrozen agglutinating plasma, the effect was generally enhancement of the agglutination.

MATERIALS AND METHODS

Agglutination tests

Plasmas and sera were obtained from New Hampshire Red chickens more than 2½ months of age. Some of these chickens had recovered from *P. lophurae* infections and others had not been infected. The low incidence of natural isoagglutinins in these chickens rendered immunization necessary. Immunization was accomplished by two to five intravenous or intramuscular injections of saline-washed erythrocytes from other chickens. Intravenous injections were made into the wing veins; intramuscular ones into the breast muscles. Each injection consisted of the suspended erythrocytes from 1.7 to 20 cc. of chicken blood.

Blood was drawn from either the right jugular or wing veins. Sodium citrate was used as the anticoagulant when plasma or erythrocytes were desired. Plasma and cells were separated by centrifugation. Cells were washed at least three times with saline, and then suspended in saline to ten times the original volume of the blood from which they were obtained, when used as the antigen in hemagglutination tests. Blood for serum was allowed to clot at room temperature or in an oven at 35°C., and then refrigerated for several hours or overnight. The serum was separated by centrifugation and the clot discarded.

Freezing of plasmas or sera was done in the freezing compartment of the refrigerator or in the freezer. Temperatures available ranged from −5 to −28°C. In the few experiments in which heated plasma or serum was used, the plasma or serum was heated at 56°C. for 30 to 90 minutes. The agglutination tests were conducted at room temperatures.

Nearly all of the agglutination tests were conducted in 10 x 75 mm. pyrex tubes. Quantities of reagents used were such that the total volume in each tube was 0.8 cc. One-fourth of this amount was cell suspension. The fraction of the 0.8 cc. that was not plasma or cell suspension was in all cases saline. The saline used in most of the tests was 0.85 per cent NaCl in distilled water.

In the experiments in which the effects of supplements of other plasmas

upon the agglutination were tested, the plasmas were mixed and generally allowed to stand at room temperature for an hour prior to addition of the cell suspension. The plasmas used as supplements were in most cases tested for ability to agglutinate the cell suspension.

In all the tests, the tubes were shaken by hand or mechanically for 3 to 20 minutes after addition of the cell suspension, and then allowed to stand undisturbed except for the taking of readings. Readings were taken at different times during the course of the agglutination. In many of the tests, the cells were resuspended after several readings, shaken again, and a subsequent reading made.

Only macroscopic agglutination was recorded in most of the tests, but the microscope was used in a few instances. The degree of macroscopic agglutination was expressed on an arbitrary scale that ranged from 0 to 8×. Titers were defined as the highest dilutions of plasma or serum bringing about 1× agglutination.

Protection tests

The strain of *P. lophurae* employed was the same one used in previous investigations in this laboratory (Becker, Brodine, and Clappison, 1949). It was maintained by serial transmission through chickens during most of the present investigations. New Hampshire Red chicks served as hosts in all the series except Series 1. White Cross chicks were used in this series. The chicks were obtained from nearby hatcheries on the day of hatching or the following day, and were infecetd when nine to 19 days old by inoculation with saline-washed parasitized chicken erythrocytes.

Plasmas were from New Hampshire Red chickens more than two months of age. Many of these chickens were used in both agglutination tests and protection tests. All of the plasma chickens used in any one series were of the same hatching. Plasmas were obtained as described under agglutination tests. The plasmas used in most of the series were stored frozen without the addition of preservative. In a few series, the plasmas were stored unfrozen after the addition of 1 cc. of phenyl mercuric nitrate solution (167 mg. in 200 cc. distilled water) to each 40 cc. of plasma as a preservative. For Series 10, the plasmas were stored unfrozen for short periods of time without the addition of preservative.

All injections were intravenous, generally into one of the wing veins. The quantitites of parasitized cells and plasma or saline injected were scaled according to the weight of the recipient. They are expressed as quantities per 100 g. of body weight. The absolute numbers of parasitized cells were only approximated, but all the chicks in a given series received the same relative quantities.

Thin blood smears were made daily until peak parasitemias were passed, and less frequently thereafter, except in Series 6. The low early parasitemias in this series rendered daily smears unnecessary until day 8. The smears were fixed in methyl alcohol, dried, and stained with Giemsa stain. The counting procedure (Becker *et al.*, 1950) and the method of determining statistical significance (Becker, Brodine, Marousek, and Byrd, 1949) have been described in previous papers.

RESULTS

Agglutination tests

Isohemagglutinins were evident in the plasmas or sera of 15 out of 21 chickens after two injections of nonparasitized chicken erythrocytes, and in the plasmas of three out of four chickens after two injections of parasitized (*P. lophurae*) chicken erythrocytes. They were also found in the plasmas of six out of seven chickens which had received more than two injections of nonparasitized chicken erythrocytes.

In contrast, isohemagglutinins were demonstrated in the plasmas of only two of 21 chickens which had not received injections of chicken erythrocytes. One of the two showing such agglutinins was in a group of 14 chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes. The other was in a group of seven chickens which had never been infected with malaria.

Effect of freezing upon the agglutination. The results of 33 agglutination tests conducted with plasmas or sera from 24 different chickens are summarized in Table 1. Where two or more different results are recorded for the same chicken, the tests were conducted on different occasions and are listed chronologically. Duplicate tests conducted at the same time are not recorded separately. The readings recorded in the "Strength of agglutination" column are for plasma or serum dilutions and times at which the greatest differences between the strengths of agglutination by unfrozen and frozen plasma or serum were observed.

Considering only chickens whose plasmas or sera (frozen or unfrozen) contained demonstrable agglutinins, it is to be seen that freezing increased the agglutinating titer of plasma or serum, or both, from 10 of the 14 chickens tested for agglutinating titer. The other four had similar titers unfrozen and frozen, but agglutination was much stronger with the frozen plasma in three out of the four instances. Six of the 24 chickens did not show demonstrable agglutinins. Freezing did not affect the lack of reactivity of plasmas from these chickens.

Freezing increased the strength of agglutination by a magnitude of 4× or more with agglutinating plasmas from 16 of 18 chickens. All 16 of these chickens had been immunized with erythrocytes from other chickens. Both cases of little or no enhancement of agglutinating power by freezing were with plasmas containing natural agglutinins. The two chickens from which these plasmas were obtained had never been injected with erythrocytes from other chickens. With one of these plasmas, freezing slightly enhanced agglutination, but with the other there was no discernible effect on either of two occasions.

Heating at 56°C. for 30 to 90 minutes also increased agglutinating titer and intensity. Results from three such experiments are included in Table 1. The effect of heating was not so great as the effect of freezing, however.

Freezing also increased the agglutinating ability of seven mixtures of three or more agglutinating plasmas which were tested with and without prior freezing. There was a pronounced effect in every instance.

TABLE 1

Effect of freezing and heating upon chicken isoagglutinating plasmas and sera

PLASMA OF CHICKEN NO.	TITER OF FRESH PLASMA	TITER OF FROZEN PLASMA	TITER OF HEATED PLASMA	STRENGTH OF AGGLUTINATION		
				Fresh plasma	Frozen plasma	Heated plasma
1	1/4	1/16	—	0	8×	—
	1/2	1/8	—	0	7×	—
1*	0	1/8	—	0	8×	—
2	1/2	1/8	—	0	8×	—
	0	1/2	—	0	5×	—
3	—	—	—	1×	5×	—
	—	—	—	1×	6×	—
4	0	0	—	0	0	—
5	—	—	—	1×	8×	—
6†	1/2	1/2	—	3×	3×	—
	—	—	—	3×	3×	—
7†	—	—	—	±	1×	—
8	1/16	1/32	—	0	8×	—
	1/4	1/32	1/8	1×	8×	6×
9	1/8	1/32	—	0	8×	—
10	0	0	—	0	0	—
11	1/2	1/8	—	0	8×	—
12	0	0	—	0	0	—
13	1/4	1/4	—	1×	7×	—
14	1/2	1/8	—	±	8×	—
15	1/16	1/16	—	±	8×	—
	—	—	—	1×	7×	—
16	1/4	1/16	—	±	8×	—
17	‡	1/4	—	±	8×	—
18	0	0	—	0	0	—
19	0	0	—	0	0	—
20	1/16	1/16	—	1×	8×	—
21	0	0	—	0	0	—
22	—	—	—	1×	7×	—
23*	—	—	—	1×	8×	6×
	1/16	1/128	—	1×	8×	—
24*	1/32	1/64	—	1×	8×	—
	1/4	1/32	1/32	±	8×	4×

* Serum.

† Natural antibodies.

‡ ± agglutination at one-half strength.

Effect of supplements of other plasmas upon the agglutination. Table 2 shows the results of a number of tests involving frozen and unfrozen agglutinating and supplemental plasmas. It is to be seen that supplements of previously frozen plasmas enhanced agglutination with unfrozen agglutinating plasmas in 19 out of 22 tests, while supplements of unfrozen plasmas enhanced such agglutination in only eight of 23 tests. Frozen supplements, then, were more effective in enhancement of agglutination by these unfrozen agglutinating plasmas. With previously frozen agglutinating plasmas, supplements of unfrozen plasmas in-

TABLE 2

Effects of supplements of other plasmas upon isoagglutination of chicken erythrocytes. (Numbers of tests in which effects were observed)

EFFECT OBSERVED	AGGLUTINATING PLASMA UNFROZEN		AGGLUTINATING PLASMA FROZEN	
	Other plasma unfrozen	Other plasma frozen	Other plasma unfrozen	Other plasma frozen
More agglutination	8	19	0	2
Less agglutination	2	1	13	13
No effect	13	2	3	4

hibited agglutination in 13 out of 16 tests and supplements of frozen plasmas inhibited agglutination in 13 out of 19 tests. The effects of frozen and of unfrozen supplements upon frozen agglutinating plasmas were, accordingly, similar. The two instances of increased agglutination with frozen agglutinating plasmas and frozen supplemental plasmas are believed to have occurred because of insufficient freezing of the agglutinating plasmas.

The effects of supplemental plasmas were similar regardless of whether they came from the chicken whose cells were being used as agglutinogen or from other chickens. Even duck plasma acted similarly to chicken plasma in a number of instances.

Effect of absorption of immune plasmas with nonparasitized erythrocytes upon their ability to agglutinate parasitized erythrocytes. Microscopic examination was used in a number of instances to determine whether or not both parasitized and nonparasitized erythrocytes were agglutinated. Plasmas from chickens that had been immunized with parasitized erythrocytes, as well as plasmas from chickens that had been immunized with nonparasitized erythrocytes, apparently agglutinated both parasitized and nonparasitized erythrocytes equally well.

Separate portions of a mixed agglutinating plasma from chickens that had been immunized with nonparasitized erythrocytes were absorbed with erythrocytes from each of three noninfected chickens. These three chickens were then infected with *P. lophurae*, and upon attainment of sufficiently high parasitemias, erythrocytes from each of the three were tested with their respective absorbed plasmas. No agglutination of either parasitized or nonparasitized cells was observed. A mixed agglutinating plasma from chickens that had been immunized with parasitized erythrocytes was also absorbed with nonparasitized erythrocytes. It failed to agglutinate either parasitized or nonparasitized erythrocytes after this absorption. Absorption with nonparasitized erythrocytes, accordingly, was effective in removing antibody capable of agglutinating parasitized erythrocytes.

Protection tests

In Tables 3 to 6, the age of the chicks when infected by inoculation with parasitized chicken red cells, the number of parasitized cells injected per 100 g. of chick, and the quantities of plasma or saline injected per 100 g. of chick follow the titles. Day 0 is the day of inoculation with the infecting dose of par-

TABLE 3

Percentages of parasitized erythrocytes, series 3 (age, 11 days; 1.5 × 10^7 parasitized cells; 0.6 cc. days 0–7)

GROUP	NO. OF CHICKS	DATA	TIME AFTER INOCULATION								OBSERVED PEAK
			1 day	2 days	3 days	4 days	5 day	6 days	7 days	10 days	
1*	8	M	0.11	0.24	2.0	3.4	9.0	3.8	0.6	0	9.9
		SD ±	0.06	0.11	0.84	1.6	6.2	5.1	1.5		5.9
2†	8	M	0.13	0.42	3.3	6.1	21.8	16.9	1.3	0	23.6
		SD ±	0.07	0.07	0.46	1.6	7.6	12.6	1.4		8.9
3‡	8	M	0.11	0.28	1.7	3.6	11.2	7.9	2.3	0	13.8
		SD ±	0.07	0.16	1.21	2.0	9.3	10.1	5.3		10.4
4§	8	M	0.12	0.44	4.2	5.9	26.8	34.4	13.0	1.7	36.9
		SD ±	0.04	0.12	0.88	1.0	4.0	15.7	13.7	3.4	11.6
5**	8	M	0.13	0.55	4.4	6.3	25.3	35.9	14.4	7.4	35.9
		SD ±	0.07	0.08	0.74	3.0	4.9	10.6	9.8	10.4	10.6

* Injected with plasma from chickens immunized with nonparasitized chicken erythrocytes.

† Injected with plasma from chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes.

‡ Injected with plasma from chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes and had then been immunized with nonparasitized chicken erythrocytes.

§ Injected with normal chicken plasma.

** Injected with saline.

asitized red cells. M is the mean and SD the standard deviation. Values below 0.01 per cent are recorded as +. Any number in the text preceded by ± is a standard deviation.

Effect of anti-erythrocytic antibodies. Series 1 consisted of three groups of eight chicks each. These chicks received 0.6 cc. injections on days 0 to 4 and 0.4 cc. injections on days 5 to 7. The injections on day 0 were administered 2 hours prior to inoculation. Group 1 received plasma pooled from two chickens which had been infected with *P. lophurae* by inoculation with parasitized duck erythrocytes and subsequent to their recovery had been injected with chicken erythrocytes. One of these two chickens had received three injections of chicken erythrocytes; the other, four injections. Group 2 received plasma pooled from two chickens which had also recovered from *P. lophurae* infections induced with parasitized duck erythrocytes, but had not been injected with chicken erythrocytes. Both plasmas had been stored unfrozen after addition of preservative. Group 3 received saline. The parasite inoculum was 5 × 10^7 parasitized cells.

Both of the plasmas conferred some passive protection, but the parasite counts of Group 1 were lower than those of Group 2. The observed peak parasitemias of the Group 1 chicks averaged 4.7 ± 5.4 per cent; those of the Group

TABLE 4

Percentages of parasitized erythrocytes, Series 4 (age, 12 days; 1.5 × 10^7 parasitized cells; Groups 1 and 2, 0.6 cc. days 0–6; Groups 3 and 4, 0.6 cc. days 0–5)

GROUP	NO. OF CHICKS	DATA	TIME AFTER INOCULATION								OBSERVED PEAK
			1 day	2 days	4 days	5 days	6 days	7 days	8 days	9 days	
1*	8	M	0.17	1.00	12.9	28.2	52.6	61.2	35.2	24.2	64.8
		SD ±	0.06	0.27	3.7	7.0	8.9	16.2	20.3	20.8	10.4
2†	8	M	0.22	0.84	10.5**	19.4**	43.6**	50.6**	34.0††	15.1††	51.4**
		SD ±	0.08	0.08	1.4	3.0	8.3	5.3	7.8	9.6	5.2
3‡	8	M	0.18	0.86	9.2	17.6	24.7	15.7	3.7	0.06	29.5
		SD ±	0.08	0.18	2.1	7.0	13.9	23.0	6.7	0.09	18.6
4§	8	M	0.17	0.75	7.7	13.3	21.8	12.8	1.8	0.01	24.0
		SD ±	0.05	0.19	1.7	3.3	16.1	15.4	4.3	0.03	13.3

* Injected with saline.

† Injected with normal chicken plasma.

‡ Injected with plasma from chickens which had been infected and once superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes.

§ Injected with plasma from chickens which had been inoculated twice with nonparasitized chicken erythrocytes.

** Seven chicks alive.

†† Six chicks alive.

TABLE 5

Percentages of parasitized erythrocytes, Series 6 (age, 11 days; 3 × 10^7 parasitized cells; 0.6 cc. days 0–2, 0.4 cc. days 3–5)

GROUP	NO. OF CHICKS	DATA	TIME AFTER INOCULATION									OBSERVED PEAK
			1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	10 days	
1*	7	M	0.30	0.58	3.4	14.3	15.7	11.7	2.1	0.4	0	18.5
		SD ±	0.15	0.29	1.8	6.3	8.7	7.8	2.7	0.8		6.5
2†	7	M	0.02	0.07	0.21	0.9	1.4	2.7	6.1	4.3	1.1	7.9
		SD ±	0.04	0.15	0.52	2.0	1.8	2.4	5.4	4.6	1.8	3.9
3‡	7	M	0.04	0.12	0.28	2.5	3.6	11.5	14.3	7.8	1.0	20.2
		SD ±	0.04	0.11	0.23	2.2	3.4	10.5	12.4	5.3	2.5	11.0
4§	7	M	0.10	0.22	1.1	5.7	11.5	22.0	19.9	8.6	0.03	28.7
		SD ±	0.08	0.20	0.9	3.3	7.5	12.8	17.0	13.3	0.07	14.6

* Injected with plasma that had been absorbed with residues from lysed nonparasitized cells.

† Injected with nonabsorbed plasma.

‡ Injected with plasma that had been absorbed with intact nonparasitized cells.

§ Control; no plasma.

TABLE 6

Percentages of parasitized erythrocytes, Series 9 (age, 11 days; 10^7 parasitized cells; for plasma dosages, see footnotes)

GROUP	NO. OF CHICKS	DATA	TIME AFTER INOCULATION										OBSERVED PEAK
			2 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	12 days	13 days	
1*	8	M	0.01	0.05	0.3	0.3	0.5	1.2	7.7	14.0	22.7**	9.2**	29.2
		SD ±	0.02	0.14	0.7	0.5	0.2	0.4	5.0	12.3	17.6	10.7	15.1
2†	8	M	0.01	0.08	0.2	1.0	3.4	8.4	8.4	6.1	+	0	13.9
		SD ±	0.02	0.16	0.4	1.7	5.3	11.0	10.5	9.1			14.0
3‡	8	M	0.18	1.8	9.2	11.3	12.9	11.2	6.7	5.5	5.2	0.6	23.6
		SD ±	0.17	1.3	6.7	13.3	21.0	18.3	7.2	7.3	11.2	1.0	16.6
4§	8	M	0.14	1.4	8.3	16.1	23.8	20.8	18.9	11.5	12.4	4.1	41.8
		SD ±	0.17	1.3	9.1	14.7	19.3	21.7	20.8	11.9	22.7	10.3	16.7

* Injected with 0.6 cc. plasma on days 0 and 1 only.

† Injected with 0.6 cc. plasma on days 0–8.

‡ Injected on day 2 with 0.6 cc. of plasma per 100 g. of combined weights for days 0–2, followed by 0.6 cc. injections on basis of daily weights on days 3–8.

§ Control; no plasma.

** Seven chicks alive.

2 chicks, 12.0 ± 6.3 per cent; and those of the Group 3 chicks, 27.4 ± 18.9 per cent. The effect of the plasma from chickens immunized against both *P. lophurae* and chicken erythrocytes was greater than that of the plasma from chickens immunized against *P. lophurae*, but not against chicken erythrocytes. Statistically, the mean peak parasitemias all differed significantly at the 5 per cent level of probability.

In Series 2, protection was first obtained with plasma from chickens which had been immunized with chicken erythrocytes, but had never been infected with *P. lophurae*. This series consisted of two groups of eight chicks each and one group (Group 3) of seven chicks. These chicks received 0.6 cc. injections on days 0 to 4 and 0.4 cc. injections on days 5 to 7. The injections on day 0 were administered 2 hours prior to inoculation. Group 1 received plasma pooled from three chickens which had received two injections of nonparasitized chicken erythrocytes, but had never been infected with malaria. Group 2 received normal chicken plasma pooled from three chickens. Both plasmas had been stored unfrozen after addition of preservative. Group 3 received saline. The parasite inoculum was 5×10^7 parasitized cells.

The chicks of Group 1 exhibited less severe parasitemias than did the controls. The observed peak parasitemias of the Group 1 chicks averaged 16.0 ± 7.3 per cent; those of the Group 2 chicks, 22.4 ± 6.8 per cent; and those of the Group 3 chicks, 28.5 ± 14.2 per cent. The mean peak parasitemias of Groups 1 and 3 differed significantly at the 5 per cent level of probability. The parasitemias exhibited by the chicks in Group 2 did not differ significantly from those of the controls.

All the treatments of Series 1 and Series 2 were represented in Series 3. This series is summarized in Table 3. Plasma and saline injections on day 0 were administered 2 hours prior to inoculation. Group 1 received eight daily injections of plasma pooled from three chickens which had received two injections of nonparasitized chicken erythrocytes, but had never been infected with malaria. Group 2 received eight daily injections of plasma pooled from three chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes, but had never been injected with chicken erythrocytes. Group 3 received eight daily injections of plasma pooled from three chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes and had subsequently been given two injections of nonparasitized chicken erythrocytes. Group 4 received eight daily injections of normal chicken plasma pooled from three chickens. These plasmas were stored frozen. Group 5 received saline.

The chickens from which the plasmas used in Series 3 were obtained were of a different hatching from the plasma donors for either Series 1 or Series 2. Plasma from the same chicken was not present in any two of the pooled plasmas used in these three series.

The parasitemias exhibited by the first three groups of Series 3 were significantly lower than those of Groups 4 and 5. The mean peak parasitemias of Groups 1 and 3 each differed significantly at the 1 per cent level of probability from the mean peak parasitemia of Group 5. The mean peak parasitemia of Group 2 differed significantly from that of Group 5 at the 5 per cent level of probability. The mean peak parasitemia of Group 1 differed significantly at the 1 per cent level from that of Group 2, while that of Group 3 did not quite differ significantly at the 5 per cent level from that of Group 2.

Protection, then, was at least as good with plasma from chickens immunized only with erythrocytes from other chickens as with plasma from chickens immunized with both *P. lophurae* and erythrocytes from other chickens. Protection with plasma from chickens immunized with *P. lophurae* but not with chicken erythrocytes was of a lower magnitude than that conferred by either of the plasmas from chickens immunized with chicken erythrocytes. No protection whatsoever was evident with normal chicken plasma.

Protection afforded by plasmas from chickens infected and superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes and from chickens similarly inoculated with nonparasitized chicken erythrocytes was compared in Series 4 and Series 5. Series 4 is summarized in Table 4. Plasma and saline injections on day 0 were administered 2¼ hours prior to inoculation. Group 1 received seven daily injections of saline. Group 2 received seven daily injections of normal chicken plasma pooled from five chickens. Group 3 received six daily injections of plasma pooled from four chickens which had been infected with *P. lophurae* by inoculation with parasitized chicken erythrocytes and had been superinfected once subsequent to their recovery by inoculation with parasitized chicken erythrocytes. Group 4 received six daily injections of plasma pooled from four chickens which had been inoculated twice with nonparasitized chicken erythrocytes. The plasmas were stored frozen.

The mean peak parasitemias of Groups 2, 3, and 4 each differed significantly from that of Group 1 at the 1 per cent level of probability. The mean peak parasitemias of Groups 2 and 3 differed significantly at the 5 per cent level of probability, while those of Groups 2 and 4 differed significantly at the 1 per cent level. The parasitemias of Groups 3 and 4 were comparable throughout the course of the infection.

Protection was at least as good, then, with plasma from chickens inoculated twice with nonparasitized chicken erythrocytes as with plasma from chickens similarly inoculated twice with parasitized chicken erythrocytes. The protection afforded by either of these plasmas was considerably better than that afforded by normal chicken plasma. Of the three series in which normal chicken plasma was tested, this was the only one in which significant protection was obtained.

Series 5 is essentially a repetition of Series 4, with one less group. This series consisted of three groups of eight chicks each. These chicks received 0.6 cc. injections on days 0 to 7. The injections on day 0 were administered 1½ hours prior to inoculation. Group 1 received plasma pooled from four chickens which had been inoculated twice with nonparasitized chicken erythrocytes. Group 2 received plasma pooled from four chickens which had been infected with *P. lophurae* by inoculation with parasitized chicken erythrocytes and had been superinfected once subsequent to their recovery by inoculation with parasitized chicken erythrocytes. These plasmas were stored frozen. Group 3 received saline. The parasite inoculum was 1.5×10^7 parasitized cells.

Again, comparable protection was obtained with plasma from chickens inoculated twice with nonparasitized chicken erythrocytes and with plasma from chickens similarly inoculated twice with parasitized chicken erythrocytes. The observed peak parasitemias of the Group 1 chicks averaged 18.6 ± 11.2 per cent; those of the Group 2 chicks, 17.8 ± 8.2 per cent; and those of the Group 3 chicks, 62.4 ± 4.8 per cent. No significant differences between parasitemias of Groups 1 and 2 were evident at any time during the course of the infection.

The plasmas used in Series 1 to 5 can be classified into four categories as follows: 1) plasmas from chickens immunized against both *P. lophurae* and erythrocytes from other chickens; 2) plasmas from chickens immunized against *P. lophurae*, but not against erythrocytes from other chickens; 3) plasmas from chickens immunized against erythrocytes from other chickens, but not against *P. lophurae*; 4) normal chicken plasmas, from chickens not immunized against *P. lophurae* or erythrocytes from other chickens.

Passive protection was effected in every one of the five experiments with every immune plasma tested. Plasmas from chickens immunized against erythrocytes from other chickens, but not against *P. lophurae*, were tested in four of these experiments and were effective in all four. In the three experiments in which such plasmas and plasmas from chickens immunized against *P. lophurae* as well as erythrocytes from other chickens were both tested, comparable protection was obtained with both.

The effect of absorption with nonparasitized chicken erythrocytes upon the

protective action of plasma from chickens infected and superinfected by inoculation with parasitized chicken erythrocytes was studied in Series 6 and 7. Series 6 is summarized in Table 5. Plasma injections on day 0 were administered 1⅓ hours prior to inoculation. The plasma used was pooled from eight chickens which had been infected and once superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes. The pooled plasma was divided into three 30 cc. portions. It was stored frozen overnight prior to absorption and was stored frozen after absorption. For the absorptions, a total of 20 cc. of blood was collected from six noninfected chickens. The cells from half of this mixture were washed once with saline, then lysed with distilled water. The residues were washed five times with saline. They were then mixed with 30 cc. of plasma and the mixture incubated for one hour at room temperature (80°F.), then kept in the refrigerator for 1½ hours. The residues were removed by centrifugation and the plasma refrozen. Group 1 received six daily injections of this plasma. Group 2 received six daily injections of nonabsorbed plasma. This plasma had been treated similarly to the plasma which Group 1 received, except that a small amount of saline had been added in place of the erythrocyte residues. Group 3 received six daily injections of plasma which had been absorbed with intact nonparasitized erythrocytes. In the preparation of this plasma, the other half of the mixture of nonparasitized erythrocytes was washed four times with saline. These washed cells were then used to absorb 30 cc. of plasma under the same conditions as the absorption with the residues from lysed cells. Group 4, the controls, received neither plasma nor saline.

The nonabsorbed plasma conferred significant protection, but the absorbed plasmas did not. The mean peak parasitemias of Groups 2 and 4 differed significantly at the 2 per cent level of probability. The mean peak parasitemias of Groups 1 and 3 did not differ significantly from that of Group 4, even at the 10 per cent level. The mean peak parasitemia of Group 1 differed significantly from that of Group 2 at the 1 per cent level, and that of Group 3 differed significantly from that of Group 2 at the 5 per cent level.

During the early stages of the infection, Group 1 exhibited higher parasitemias than did Group 4. On day 1, for example, the mean parasitemias of these groups differed significantly at the 2 per cent level of probability. The effect of the plasma was apparently the reverse of protection.

Series 7 consisted of three groups of 11 chicks each. These chicks received 0.6 cc. injections on days 0 to 5. The injections on day 0 were administered 1⅔ hours prior to inoculation. The plasma used was pooled from nine chickens which had been infected and superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes. Seven of these nine chickens had been superinfected twice and the other two had been superinfected once. Group 1, the controls, received saline. Group 2 received nonabsorbed plasma. Group 3 received plasma which had been absorbed with washed residues from lysed nonparasitized erythrocytes pooled from ten chickens. The residues from 20 cc. of blood were incubated with 55 cc. of plasma for one hour at room temperature and then for ½ hour in the refrigerator. The plasma was kept frozen overnight

prior to absorption. All plasmas were stored frozen. The parasite inoculum was 3×10^7 parasitized cells.

Unlike Series 6, incubation of the plasma with residues from lysed nonparasitized erythrocytes did not bring about a loss of its protective ability. The parasite counts of Group 3 were actually lower than those of Group 2, but the difference was not significant. The observed peak parasitemias of the Group 1 chicks averaged 24.0 ± 8.9 per cent; those of the Group 2 chicks, 13.5 ± 6.8 per cent; and those of the Group 3 chicks, 8.7 ± 4.5 per cent. The peaks of Groups 2 and 3 each differed significantly from that of Group 1 at the 1 per cent level of probability, but did not differ significantly from each other at the 5 per cent level.

Effect of time of administration of plasma. In the previous five series, the plasmas were always administered for at least six consecutive days, the first plasma injection being prior to the injection of the infecting inoculum of parasitized chicken erythrocytes. Protection could also be obtained when plasma injections were begun 2 days after inoculation with parasitized erythrocytes. Since the life cycle of *P. lophurae* is approximately 36 hours in length (Hewitt, 1949), the parasites are all in the recipient's own erythrocytes two days after infection.

Series 8 consisted of three groups of ten chicks each. The plasma used in this series was pooled from six chickens which had received two injections of nonparasitized chicken erythrocytes. Two of these six chickens had previously recovered from *P. lophurae* infections induced with parasitized duck erythrocytes. The plasma was stored frozen. Group 1 received 1.8 cc. of saline on day 2, 0.4 cc. on days 3 to 12, and 0.3 cc. on day 13. Group 2 received 1.8 cc. of plasma on day 2, 0.4 cc. on days 3 to 12, and 0.3 cc. on day 13. Group 3 received 14 daily injections of plasma, the first 2 hours prior to inoculation on day 0. This group received 0.6 cc. on days 0 to 2, 0.4 cc. on days 3 to 12, and 0.3 cc. on day 13. The total volumes of plasma or saline per unit of body weight received by each of the groups were approximately the same. The parasite inoculum was 10^6 parasitized cells.

The parasitemias exhibited by Groups 2 and 3 were both significantly lower than those of Group 1. The observed peak parasitemias of the Group 1 chicks averaged 27.5 ± 19.1 per cent; those of the Group 2 chicks, 7.5 ± 13.3 per cent; and those of the Group 3 chicks, 1.0 ± 1.1 per cent. The mean peak parasitemias of Groups 1 and 2 differed significantly at the 2 per cent level of probability, while those of Groups 1 and 3 differed significantly at the 1 per cent level. The mean peak parasitemias of Groups 2 and 3 did not differ significantly at the 5 per cent level or even at the 10 per cent level. The mean parasitemias of these groups did not differ significantly at any stage of the infection.

Series 9 is summarized in Table 6. The plasma used was pooled from six chickens which had received three injections of nonparasitized chicken erythrocytes, but had never been infected with malaria. This plasma was stored frozen. Group 1 received 0.6 cc. of plasma on days 0 and 1 only. Group 2 received 0.6 cc. of plasma on days 0 to 8. The plasma injections on day 0 were administered

1¾ hours prior to inoculation. On day 2 Group 3 received 0.6 cc. of plasma per 100 g. of combined weights for days 0 to 2. On days 3 to 8 this group, like Group 2, received 0.6 cc. injections based on daily weights. Groups 2 and 3, accordingly, received the same total dosages of plasma. The control group (Group 4) received neither plasma nor saline.

The parasite counts for Groups 1 and 2 were comparable through day 9. Although the means for these groups differed considerably on days 6 to 8, these differences were due to the high parasitemias of only two of the chicks in Group 2. The mean parasitemia of Group 1 continued to rise through day 12, while that of Group 2 declined after day 9. The difference between the two groups was most marked on day 12, when the parasitemias of the chicks in Group 1 averaged 22.7 per cent, in contrast to the 0.004 per cent for the Group 2 chicks.

During the early stages of the infection, the parasite counts of Groups 3 and 4 were comparable and higher than those of Groups 1 and 2. Later, however, the Group 4 chicks exhibited more severe parasitemias than did those in Group 3.

The mean peak parasitemias of Groups 2 and 4 differed significantly at the 1 per cent level of probability, while those of Groups 3 and 4 differed significantly at the 5 per cent level. The mean peak parasitemias of Groups 1 and 2 differed significantly at the 10 per cent level, but not at the 5 per cent level. No other comparisons of mean peak parasitemias showed any significant differences, even at the 10 per cent level. Protection statistically significant at the 5 per cent level of probability was obtained, then, only when the plasma was administered on days 0 to 8, or an equivalent amount of plasma was administered starting on day 2.

Effect of freezing. In view of the marked effect of freezing upon the *in vitro* agglutinating ability of isoimmune chicken plasma, it was desirable to determine whether such treatment would also affect the *in vivo* protective ability of such plasma. Unfrozen and frozen isoimmune plasmas were tested simultaneously in Series 10. This series consisted of three groups of eight chicks each. These chicks received 0.6 cc. injections on days 0, 2, 4, and 6; and 0.4 cc. injections on days 1, 3, and 5. The injections on day 0 were administered 2 hours prior to inoculation. Group 1 received unfrozen plasma. Group 2 received previously frozen plasma. Group 3 received saline. The plasmas used were from five chickens which had received three injections of nonparasitized chicken erythrocytes, but had never been infected with malaria. Three chickens were bled and their plasmas pooled on each of the following days: 0, 2, 4, and 6. The first three pooled plasmas were injected on the day they were collected and on the following day; the last was injected only on the day collected. Thus, no plasma was more than one day old when injected. Half of each pooled plasma was frozen and thawed, the other half kept unfrozen. The frozen plasmas were kept in the freezer for 2 hours or less and were then thawed and kept unfrozen until use. Samples of unfrozen and frozen plasma from each pool were tested in agglutination tests. In every case stronger agglutination was obtained with the previously frozen plasma. The parasite inoculum was 3×10^7 parasitized cells.

Comparable protection was obtained with the unfrozen and frozen plasmas. The parasitemias exhibited by the chicks in Group 1 were similar to those exhibited by the Group 2 chicks. The observed peak parasitemias of the Group 1 chicks averaged 0.38 ± 0.30 per cent; those of the Group 2 chicks, 0.29 ± 0.13 per cent; and those of the Group 3 chicks, 29.8 ± 16.3 per cent. Marked protection was evident in this experiment.

DISCUSSION

The mechanisms of the effects of freezing and of supplements of other plasmas upon isohemagglutinating chicken plasmas are unknown. The effect of freezing may very well be similar in nature to the effect of standing upon chicken anti-mammalian serum protein sera observed by Wolfe (1942) and Wolfe and Dilks (1946). These workers found that the precipitin titers of such sera increased upon standing under refrigeration, maximum titers generally being reached within five to eight days after bleeding. Antisera produced in rabbits, however, did not increase in titer upon standing. Wolfe (1942) postulated that increase in titer upon standing may have been due to an inhibitory substance present in the fresh sera losing its activity upon standing. Becker *et al.* (1952) similarly postulated that destruction of a cryolabile inhibitor was responsible for enhanced agglutination of duck erythrocytes by chicken plasmas after freezing and thawing of the chicken plasmas.

Statistically significant protection with normal chicken plasma was observed in only one of three experiments in which such plasmas were tested. Even in this experiment where normal chicken plasma had some protective effect, it was not nearly as effective as the other plasmas tested. In view of the results obtained in these three experiments, it seems reasonable to believe that the effects of the other plasmas were not due merely to the presence of natural antibodies not induced by the malaria or injected chicken erythrocytes.

The evidence is believed to indicate that much if not all of the protective effect of plasma from chickens infected and superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes is due to antibodies induced not by the parasites, but by the injected erythrocytes. Plasmas from chickens inoculated only with nonparasitized chicken erythrocytes were fully as protective as plasmas from chickens infected and superinfected with this species of malaria parasite by inoculation with parasitized chicken erythrocytes.

True anti-malarial antibody may also play a role in the protective action of plasma or serum from chickens infected and superinfected by inoculation with parasitized chicken erythrocytes. In Series 1 and 3, some protection was obtained with plasmas from chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes, but had never been injected with chicken erythrocytes. The protective action of plasma or serum from chickens infected and superinfected by inoculation with parasitized chicken erythrocytes might be due to both antierythrocytic and antimalarial antibodies. Or, the protection obtained with plasma from chickens recovered from infections induced

with parasitized duck erythrocytes could possibly be due to anti-duck erythrocyte antibody, but this is doubtful.

Further evidence of the role of anti-erythrocytic antibodies is found in the results of the absorption tests, with the exception of Series 7. Absorption with nonparasitized erythrocytes rendered plasmas incapable of agglutinating parasitized erythrocytes and of conferring significant protection against *P. lophurae*. Zuckerman (1945), Coffin (1951), Stauber and Walker (1946) and Stauber *et al.* (1951) have reported similar findings. The reason for the failure of incubation with nonparasitized erythrocyte material to affect the protective ability of the plasma used in Series 7 is not known. The results obtained in this series may be cited as evidence against the role of anti-erythrocytic antibodies. The technique used in carrying out the absorption may have been responsible. Time did not permit a third experiment of this nature.

That the injected erythrocytes rather than the malarial parasites are the stimulus for the production of the anti-erythrocytic antibodies is further substantiated by the observation of Zuckerman (1945) that such antibodies were found in the sera of chickens infected and superinfected by inoculation with parasitized chicken erythrocytes, but not in the sera of chickens recovered from malarial infections and not superinfected. This is corroborated in the present work by the observed low incidence of such antibodies in plasmas from chickens recovered from infections induced with parasitized duck erythrocytes, in contrast to the high incidence observed in plasmas from chickens injected with nonparasitized or parasitized chicken erythrocytes. This reasoning may be objected to on the grounds that chickens which have been superinfected a number of times have received more antigenic stimulation from the parasites themselves, as well as from injected erythrocytes, than have chickens not superinfected.

Anti-erythrocytic protective antibodies were not always demonstrable by the macroscopic agglutination reaction. The plasma used in Series 6, for example, did not bring about any macroscopic agglutination of samples of the inoculum used to infect the chicks, but the protective action of this plasma could be absorbed out with nonparasitized erythrocytes.

The high parasitemias during the early stages of the infection, in comparison to the controls (Group 4), of the chicks (Group 1) in Series 6 which received plasma absorbed with residues from lysed nonparasitized erythrocytes, are reminiscent of the sparing phenomenon of Becker and his collaborators (Becker, Brodine, Marousek, and Byrd, 1949). When the experiment was repeated (Series 7), no such effect was observed. This disparity of results may have been due to faulty technique in Series 7. Conversely, the difference observed in Series 6 may have been a result of rapid degenerative changes in the parasite inoculum during standing, so that Group 1 received a more virulent inoculum than did Group 4.

The passive protection of chicks against *P. lophurae* infections with plasmas containing anti-erythrocytic antibodies is not merely the result of such anti-

bodies aiding in the destruction of the infecting inoculum of parasitized cells before the parasites have time to sporulate and infect the recipient's own cells. Evidence to support this statement is to be found in the results of Series 8 and 9. If the protection were merely the result of the plasma acting against the initial inoculum of parasitized cells, protection would have been obtained when plasma was administered on days 0 and 1 only and would not have been obtained when plasma injections were started on day 2. In Series 5, moreover, no protection whatsoever was apparent on day 2, but marked protection showed up later.

SUMMARY

1. Isohemagglutinins were demonstrated in the plasmas or sera of 24 out of 32 chickens which had been immunized with nonparasitized or parasitized chicken erythrocytes, but were evident in the plasma of only one out of 14 chickens which had recovered from *P. lophurae* infections induced with parasitized duck erythrocytes and had never been injected with chicken erythrocytes. Such antibodies were demonstrated also in plasma from one of seven normal chickens tested.

2. Freezing and thawing markedly enhanced the agglutinating powers of 16 out of 18 isoagglutinating chicken plasmas tested and slightly enhanced the agglutinating power of one of the other two.

3. Supplements of previously frozen plasmas from other chickens enhanced agglutination by unfrozen chicken isoagglutinating plasmas in 19 out of 22 tests. Supplements of unfrozen plasmas enhanced such agglutination in only eight of 23 tests. Frozen supplements, then, were more effective.

4. Supplements of previously frozen plasmas from other chickens inhibited agglutination by previously frozen chicken isoagglutinating plasmas in 13 out of 19 tests. Supplements of unfrozen plasmas similarly inhibited agglutination in 13 of 16 tests. Frozen and unfrozen supplements were equally effective.

5. Chicken isoagglutinating plasmas agglutinated both nonparasitized and parasitized erythrocytes. Absorption with nonparasitized erythrocytes rendered such plasmas incapable of agglutinating either parasitized or nonparasitized erythrocytes.

6. Passive protection against *P. lophurae* was effected with plasmas from chickens which had been immunized against erythrocytes from other chickens, but had never been infected with malaria. Such plasmas were fully as effective as plasmas from chickens which had been immunized against both *P. lophurae* and erythrocytes from other chickens. The latter chickens were inoculated with chicken erythrocytes after recovering from *P. lophurae* infections induced with parasitized duck erythrocytes, or were infected and superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes. Some protection was obtained with plasmas from chickens immunized against *P. lophurae* but not against chicken erythrocytes, but it was of a lower magnitude than that conferred by plasmas containing anti-erythrocytic antibodies.

7. In one experiment, absorption with residues from lysed nonparasitized erythrocytes and with intact nonparasitized erythrocytes resulted in loss of

protective power of plasma from chickens infected and superinfected with *P. lophurae* by inoculation with parasitized chicken erythrocytes. Chicks receiving the plasma absorbed with the residues from the lysed erythrocytes actually showed higher parasitemias during the first few days of the infection than did the control chicks. In a second experiment, absorption with residues from lysed nonparasitized erythrocytes did not alter the protective effect of a similar plasma.

8. Passive protection was obtained when administration of plasma was begun two days after inoculation, as well as when plasma injections were begun prior to inoculation. No significant protection was evident when plasma was administered for only two days.

9. Comparable protection was afforded by unfrozen and previously frozen plasmas from chickens immunized against chicken erythrocytes but not against malaria.

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THE SUPPRESSIVE TREATMENT OF MALARIA IN A RURAL VILLAGE WITH PRIMAQUINE AND PLAQUENIL

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For many years we have been trying several of the new antimalarial drugs. Our object in treating rural malaria is the saving of labor days and the improvement of school attendance rather than a full course of treatment directed at complete eradication of the parasites. We have had such good results in the mass treatment of rural villages with chloroquine (Aralen) that we have hesitated to change to primaquine or any of the newer drugs. Some writers say that the suppressive effect of chloroquine is superior to that of primaquine and that toxicity is less apparent. During the past two years Col. John R. Wood, M.C., Chairman, Medical Research and Development Board, Office of the Surgeon General, has suggested to our home office in Washington, D. C. that we try out primaquine in the dark and black skinned races. Some evidence had accumulated that these races are more sensitive to the toxic effect of the drug than others. Experience with other 8-aminoquinolines suggested that hemolytic anemia might occur if the drug were given over a long period of time.

We employed a tablet containing 30 mg. of primaquine and 300 mg. of Plaquenil, kindly furnished by Winthrop-Stearns, Inc. Plaquenil is similar to chloroquine but may be less toxic. The combined tablet saves giving two separate tablets on treatment days and facilitates the use of both types of drug. Little was known about the tolerance of children to primaquine at the start of this study. We could not afford to leave children out of the trial because our surveys for many years on rural communities have always shown that the thick blood film rates in children are almost always twice or two and a half times as high as in adults, so we naturally wanted to include all ages. The tablets were scored so that they could be easily broken in half or even in quarters. The dosage was discussed with Dr. Alf Alving, Professor of Medicine, University of Chicago, and it was his opinion that the following dosage scheme would be tolerated by the dark skinned races of all ages and would probably be effective in preventing relapses due to vivax malaria. The composition of the new tablet is shown below in terms of primaquine base and chloroquine base, to be given in a single dose each week for about six months (Table 1).

We have used half a tablet each week for those weighing about 90 pounds, a quarter of a tablet for those under this weight but above six months of age; the adults were given a whole tablet each week.

We received the drug in June, 1952, but were delayed for some time before we could find a satisfactory location for the test. We wanted a place with a high rate of malaria where no control measures were in use, such as mosquito breeding control, house sprays with DDT and the use of antimalarial drugs in the schools. We investigated 15 places within a half day's motor car travel of Panama with-

TABLE 1

Dosage of primaquine and Plaquenil used, in a combined tablet

BODY WEIGHT, lb.	PRIMAQUINE, mg.	PLAQUENIL, mg.
15 or less	5	50
15 to 45	10	100
45 to 90	15	150
90 or over	30	300

out finding a suitable place although a few years ago all of these places had a high blood-film rate.

On August 6, 1952, we surveyed Rio Indio, a village about 20 miles up the Atlantic coast from the western boundary of the Canal Zone, located at the mouth of a river by the same name. Thick blood films were made from 92 people and 49, or 52.2 per cent revealed malarial parasites: *P. falciparum* in 36, *P. vivax* in 9, *P. malariae* in 3, and a mixture of *P. falciparum* and *P. vivax* in one. There were 42 adults and 50 children under 15 years of age. Fifteen adults and 34 children were positive for parasites. Treatments and taking of blood films weekly were begun September 11, 1952. Motor car transportation was provided by the Army from Panama City to Gatun where an Army jeep was used from Gatun to Salud; from this point to Rio Indio it was a two hours' walk on a sea-coast trail.

As the weekly visits were made, the names in the field record book increased until at the close of the period there were 186 names. However these people have their farms spread out over several miles of the river valley and attendance was very irregular for many of the people because of heavy rains and the planting and harvesting periods. There were 93 people with regular attendance on treatment days over a sufficient period, although many were not treated for the entire time. We have taken for our analysis those who were treated regularly from 20 to 26 weeks (Table 2).

It took about three weeks of suppressive treatment before most of the weekly blood-film surveys were reported as negative. After October 1 only six of the

TABLE 2

Number of persons receiving drugs for each treatment period

REGULAR TREATMENTS	NUMBER OF PERSONS SO TREATED
weeks	
26	15
25	14
24	17
23	16
22	11
21	5
20	15

regularly treated individuals were found positive for parasites. They are listed as follows:

1. Eduardo Lanson, age 2 years. On August 6, Sept. 10, 18 and 25 his blood was positive for *P. falciparum*. On October 10 he was again positive for the same parasite but was negative for the rest of the period.

2. Jacinto Lanson, age 1 year. On August 6 his blood film showed *P. falciparum* parasites, and on Sept. 11 and 25 his film contained both *P. falciparum* and *P. vivax*. He later became positive for *P. falciparum* November 6, Jan. 22 and Jan. 29. We have no way of knowing how many treatments this child retained and absorbed for as a rule he would immediately regurgitate the dose. He would do the same with small pieces of candy and pieces of soft sweet milk chocolate. The mother took some of his treatments home with her and occasionally he retained a dose.

3. Berta Valdez, age 3 years. On August 6 her blood film contained *P. falciparum* parasites, on Sept. 11 a mixed infection of *P. falciparum* and *P. vivax* was found while on Sept. 25 only *P. falciparum* appeared. On October 2 and 10 *P. falciparum* parasites were present but for the rest of the period no parasites were found.

4. José de la C. Valdez, age 16 years, was negative on all of the early surveys but on February 26, 1953, *P. vivax* parasites appeared in the film.

5. Marcela Vallejos, age 8 years. The initial survey on Aug. 6 revealed *P. vivax* parasites. On Sept. 11 some parasites of *P. falciparum* appeared. October 2 a few *P. falciparum* parasites were recorded but after that date all films were negative.

6. Miguel Vallejos, age 3 years. The initial survey of Sept. 25 showed few *P. falciparum* parasites and the same species appeared in the film on October 17. All others were negative.

During the period between October 2 and March 5, of those who had received regularly from 20 to 26 weekly treatments, 5 of 38 children (13.2%) and one of 12 adults (8.3%) showed positive films.

Relapses or the reappearance of parasites during the period of treatment. There were 6 cases, 3 with *P. falciparum*, 2 *P. falciparum* and *P. vivax* and one, *P. vivax*. The last case did not reveal parasites in the blood-film surveys until very late during the six months treatment.

The suppressive use of the drug ended March 5, 1953 but another blood film survey was made 16 weeks later on June 25, 1953, to obtain further information. This survey included 95 people and 19 of them (20 per cent) had parasites in their blood films. There were 53 children with 13 positives (24.5 per cent), and 42 adults, 6 of whom were positive (14.3 per cent). Fifteen of these positives had been regularly treated for from 20 to 25 weeks. All were children except one. The species of parasites found were 8 *P. falciparum* cases and 7 *P. vivax*.

DISCUSSION

During the six months course of treatment no toxic results were noted in these dark skinned people, either in children or adults. The treatment results

were good but no better than we would have expected to get with chloroquine alone. The parasites disappeared from thick blood-films in about three weeks except for the rare individual.

A letter dated March 11, 1953 was received from Col. John R. Wood in which he states that his early apprehensions regarding the use of this combined dosage in dark skinned races were unfounded. Dr. Alving in Chicago has since used the same combination of the drugs in the same dosage used at Rio Indio on 100 Negroes for nine months in Stateville Prison, Joliet, Ill. He was unable to detect any evidence of toxicity.

SUMMARY AND CONCLUSIONS

Ninety-three dark-skinned individuals (42 adults and 51 children) living in a highly malarious village in Panama, were given suppressive treatments weekly with a combination of primaquine and Plaquenil for from 20 to 26 weeks. After the first 3 weeks, only 6 were found positive for malaria parasites on weekly examination of thick blood-films, although the percentage of positives was 52.2% at the start. No toxic results were noted either in children or adults.

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The technicians of the Gorgas Memorial Laboratory who participated in the work were: Lionel de Sousa and Néstor González. Both of these men have had many years of experience in preparing and examining such blood films.

ANTIBIOTIC SYNERGISM IN AMEBIASIS

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Despite current reports of successful use of several antibiotics against amebiasis in man, subsequent studies have not always completely confirmed first observations. In many instances, the groups of patients were dissimilar; some cases were predominantly chronic, others acute. Variations in response from 50 to 90 per cent to the same agent have been reported. The certainty of diagnosis has not always been confirmed by iron-hematoxylin stained, fixed preparations of *Endameba histolytica*. Follow-up studies not infrequently have been made for less than three months; some groups have been followed for 20 days, others for six weeks. Clinical evidence of success or failure has not always been critical. Further, patients under study often have returned to the environment where their amebic infection was first detected, and possible reinfection has not been taken into account. It is therefore not surprising that we appear to be no more successful today, despite the host of newer agents, including several antibiotics, than we were a decade ago in clearing up more than 90 per cent of chronic cases with persistent therapeutic measures.

Thus, we are faced with the necessity of utilizing studies *in vitro*, which attempt to distinguish between "direct" amebicidal activity as contrasted with "indirect" effects on associated bacteria in culture. Also, we must make use of chronic, natural amebiasis in macaques as a laboratory model of chronic, drug-refractive human amebiasis in the Western hemisphere. In addition, some information has been derived from artificially induced rodent amebiasis, an approximate counterpart of the acute fulminating disease of man. Finally, however, we must evaluate in man, under the best controlled conditions possible, those promising agents most likely to have widespread clinical use.

Currently, fumagillin, of the newer antibiotics, is the most useful of the "direct" acting agents. Evidence from studies *in vitro* and in rodents, originally reported by McCowen *et al.* (1951), and by studies of the authors and their associates *in vitro*, in macaques and in chronic, human cyst-passers (1951) and by Killough *et al.* in Egyptian patients (1951) has indicated that this is an effective antibiotic in the majority of instances. Recently, McHardy *et al.* (1953) have increased the low doses originally recommended and have reported even greater success in a carefully controlled study in which accepted amebicides were included for comparison clinically.

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TABLE 1

Laboratory studies

ANTIBIOTIC	*In vitro* ACTIVITY LEVELS*		*In vivo* ACTIVITY IN MACAQUES	
	With "streptobacillus" associate dilution	With *Trypanosoma cruzi* associate dilution	Daily dose over 10 days mg./kg.	Cleared over 3 months ratio
Fumagillin	1:8 million	1:4–1:16 million	50–200	12/19
Erythromycin	1:5000	1:1000 (not active)	25–200	0/11
Fumagillin plus Erythromycin concurrently	1:10 million + 1:10,000	1:32 million + 1:1000	12.5 + 12.5	5/7
Neomycin	1:1000	1:1000 (not active)	100–200	5/8†
Fumagillin plus Neomycin concurrently	1:40 million + 1:5000	1:100 million + 1:4000 (not active)	100 + 100	5/5†

* Emetine hydrochloride is active at 1:100,000 to 1:400,000.

† Follow-up studies in progress; no clearance with Neomycin at 100 mg./kg.

In 1952, McCowen *et al.* reported another antibiotic, having a spectrum of antibacterial activity comparable to that of penicillin, which was active *in vitro* and in rodents against *E. histolytica*. This agent, erythromycin, had been found by our studies *in vitro* to be essentially antibacterial, without direct action against *E. histolytica*. It was not comparable to fumagillin as an effective agent in monkey amebiasis. A summary of our findings is given in Table 1, comparing the more effective agents, including neomycin.

Thus, while erythromycin was not comparable to fumagillin *in vitro* or in monkey trials, it did, nevertheless, appear to synergize with fumagillin, both *in vitro* and in monkeys. These observations prompted exploration of the usefulness of combined therapy in man, in comparison with the use of each antibiotic alone under carefully controlled conditions.

It will be noted in Table 1, that there is some evidence of synergism *in vitro* between neomycin and fumagillin in the presence of a bacterial associate only. In monkeys, a dose of 200 mg. per kilo of neomycin alone is required to clear the majority of animals. When combined with fumagillin, 100 mg. per kilo of each agent given perorally cleared macaques for six weeks or longer, but 3 of 5 animals at this dose level lost 6, 13 and 25 per cent of their initial weight during the follow-up period. Thus, it would appear that we have used the highest doses of these antibiotics which can be tolerated concurrently by macaques.

At the Sonoma State Hospital in California, where the incidence of amebiasis

has been found to be relatively high (Nelson *et al.*, unpublished data) 100 children, ages 5 to 14 years (mean 8.8 years), were selected for critical clinical appraisal. The mean weight for the group approximated 22.0 (range from 13.2 to 35.8) kilos. Thirty-eight patients among this group were found to be infected with *E. histolytica*, by iron-hematoxylin staining technic. As soon as detected, each patient was isolated for the period of therapy and by random (1, 2, or 3, etc.) selection was treated according to one of the three regimes noted. On completion of therapy each child was maintained with other antibiotic-treated patients in isolation. To guard further against possible reinfection, the 62 patients not found to harbor *E. histolytica* were treated with conventional amebicides on accepted dosage schedules. Finally, the nursing personnel were established as having 3 negative stool examinations.

All of the 38 infected children and one adult in this institution for the mentally retarded were treated during 52 separate episodes of infection with *E. histolytica* as outlined below. Nine of the 38 children were considered as having frank dysentery with diarrhea, mucus and hyperactive peristalsis, and exhibited either blood in the stools or the motile form of *E. histolytica* or both. Eight other children at the time of diagnosis were having diarrhea with hyperactive peristalsis and appeared ill but did not pass gross blood or trophozoites of *E. histolytica* in their stools. Seven other children showed evidence of chronic amebiasis, with associated malnutrition and recurrent bouts of diarrhea over the several months previous to therapy. The remainder of the group (14 total) were asymptomatic and were classified as cyst passers.

One of the patients with frank dysentery and another found to be a cyst passer were found to have enlarged livers on examination by abdominal palpation. All patients under study were during the first several days of treatment given a cephalin flocculation test, and determination of icteric index. In all cases the results of these tests were found to be within normal limits. All other clinical-laboratory tests except the finding of *E. histolytica* were essentially negative.

The thirty-eight pediatric patients were treated perorally as follows:

Thirteen were given initially, combined therapy, that is, 100 mg. daily of erythromycin stearate[2] plus 10 mg. of fumagillin over 14 days. Later, 4 patients on recurrence were given 20 mg. of fumagillin plus 200 mg. of erythromycin stearate daily over 14 days; 4 other patients were given 40 mg. and 400 mg. daily doses of each antibiotic over 14 days.

Seventeen children were given 300 mg. daily of erythromycin stearate[2] alone over 14 days.

Fourteen children were given 40 mg. of fumagillin alone, daily over 14 days. In four of these patients previous therapy had not been effective. Table 2 summarizes the results. There was no consistent effect of therapy on nonpathogenic protozoa.

It is of interest, that during the 3 month period of follow-up (up to 3½

[2] In the form of suspension, supplied by the Abbott Laboratories.

TABLE 2

Clinical trials of antibiotics in 38 children having E. histolytica *infection**

ANTIBIOTIC	NUMBER OF EPISODES TREATED DAILY SCHEDULES	NUMBER OF RECURRENCES†
Fumagillin	10: 10–30 mg. daily × 18	5
	4: 40 mg. daily × 14	1
Fumagillin (F) plus Erythromycin (E)	13: 10 mg. (F) + 100 mg. (E) daily × 14	5
	4: 20 mg. (F) + 200 mg. (E) daily × 14	
	4: 40 mg. (F) + 400 mg. (E) daily × 14	0
Erythromycin	17: 300 mg. daily × 14	2

* Mean age 8.8 years; weight 22.0 kilograms.

† During three months follow-up; 10 or more specimens examined.

months), when 10 or more stained specimens were examined for each treated patient, the following recurrences were detected:

Two infections recurred among 17 patients given erythromycin alone, 6 occurred among 14 patients given fumagillin alone, and 5 among 21 patients given the two antibiotics in combination, at various dose levels. Among these patients who had recurrences of amebic infection all but one have been successfully cleared of infection before termination of the study.

Fumagillin was given in capsules if the children would take them. Otherwise, the capsules were opened and the powder was mixed with raspberry syrup and given in this form. Erythromycin was given as a suspension of the stearate salt. In all cases both antibiotics were well tolerated, even when given concurrently in higher doses. No variations were made in dosage because of age or weight of the patient. Thus, the dose on a per kilo basis for fumagillin exceeded levels where, in adults, signs and symptoms of toxicity have been reported by others (McHardy *et al.*, 1953).

In all cases initial laboratory diagnosis was made by examination of fresh saline preparations of feces and iodine or iodine plus eosin preparations. Stools suspected of showing *E. histolytica* in the fresh preparation were divided into two portions; one portion was sealed in a small bottle and the other portion preserved in Schaudinn's solution plus polyvinyl alcohol. These specimens were placed on slides and were fixed and stained by the iron-hematoxylin technic. The only patients included in this study were those having episodes in which the initial laboratory diagnosis was confirmed by examination of iron-hematoxylin stained smears. Follow-up specimens were all examined by iron-hematoxylin technic and preserved for confirmation.

Each patient in this study, after a complete physical examination at the time of diagnosis, was transferred to one of two separate isolation units within the institution for the duration of treatment and during the three months follow-up

period. Any patient found to have relapsed was immediately removed from these isolation units. In this way an attempt was made to keep reinfection at a minimum. The number and character of stools during the treatment and other pertinent data obtained during the follow-up period were charted daily. All patients were observed daily during medical and nursing rounds in the hospital where they were isolated. Specimens for follow-up examinations were taken from 10 to 25 days after completion of therapy and continued over a 3 to 3½ month period. A minimum of 10 specimens were taken from each patient after any episode where treatment was instituted.

Since patients were selected at random, the frank dysentery and diarrhea cases were divided equally among the three treatment regimes, and in most cases there was a prompt response to treatment with subsidence of acute symptoms except diarrhea. Half of the cases treated with erythromycin alone developed a mild diarrhea or passed soft stools on the 2nd or 3rd day of therapy. This was not related to the diarrhea present before treatment. The drug-induced diarrhea lasted from two to four days and then cleared spontaneously. After an initial subsidence of diarrhea, due presumably to amebic infection, five of the cases treated with fumagillin alone developed a brief, mild diarrhea during the course of treatment. This was observed also in previously asymptomatic cases. Except for this effect, which was considered to be due to transient intolerance to antibiotics, diarrhea subsided promptly and uniformly during all treatment regimes.

In all patients given either or both antibiotics in the dosage range indicated, there was no clinical, or laboratory evidence of drug toxicity in any case. The results of liver function tests in every patient and other appropriate tests were not altered by therapy. The dosage schedule for erythromycin was believed not to be excessive and in no patient was more than 30 mg. per kilo given daily. On the other hand, the largest fumagillin dosage approximated 3 mg. per kilo and exceeded by fifty per cent the range where McHardy *et al.* (1953) had noted anorexia, nausea, abdominal discomfort, diarrhea, vertigo and occasionally emesis in adults.

It should be noted that an inventory of intestinal parasites which appeared only during the follow-up period, indicated that in 8 of the 13 recurrences there were parasites which were not found before treatment. This would suggest that the possibility of fecal contamination from other infected individuals existed, despite our precautions. In any study, however well controlled, this is constantly an unknown factor. But it does not explain the less frequent recurrences in the erythromycin-treated group. Erythromycin stearate, 15 mg. per kilo of body weight, daily over 14 days, is now being given to a larger group of patients (by T. L. N.) since it apparently exhibited the smallest rate of recurrence, excepting fumagillin plus erythromycin at high dose levels. The possible role of associated bacterial flora in the pathogenesis of amebiasis must be considered. As Shookhoff has suggested, the "antibacterial" antibiotics may destroy intestinal bacteria which provide a suitable nutrient substrate for the ameba.

DISCUSSION

Despite earlier (Anderson and Anderson, 1950) attempts to demonstrate synergism among antibiotics as a more effective therapeutic device than the use of single agents, neither in monkeys nor at present in patients have we found true synergism completely effected. Thus, despite suggestive earlier results obtained *in vitro* (Nakamura *et al.*, 1953) in which fumagillin plus terramycin, and fumagillin plus thiocarbarsone, proved to be active, in the present study erythromycin and fumagillin were not significantly more active when given together than fumagillin given alone in human patients, and apparently the combination was no more active than erythromycin given alone. In macaques, however, the combined use of these antibiotics would appear to be more effective. Further, our clinical findings appear to be in agreement with only a part of the preliminary results *in vitro*. Thus, when associated bacteria are present, the role of the essentially "antibacterial" antibiotic becomes important.

The more favorable response to erythromycin stearate suspension given perorally is most surprising. One is reminded of the early promise of aureomycin (McKay *et al.*, 1950) which subsequently proved to be not so effective. In our hands, only lethal doses were effective in macaques. One is also reminded of terramycin (Most, 1950) which in our own experience in man did not confirm the original reports. In fact, this antibiotic was less effective than fumagillin in chronic cyst passers (Anderson *et al.*, 1951). Subsequent studies by Weiser *et al.*, Radke (1953) and T. L. Nelson (unpublished data) raised similar questions on the efficacy of terramycin as an amebicide. Of the essentially "antibacterial" antibiotics, according to our own observations, only erythromycin appears to

TABLE 3

*Therapeutic evaluation of amebacides in man**

	TIMES USED	THERAPEUTIC EFFICIENCY PER CENT
Emetine (never used alone)	135	—
Carbarsone (R)	468	93.9
Anayodin (R)	459	92.9
Diodoquin (R)	41	89.9
Sulfonamide derivatives	18	0
Combination of two or more drugs	297	94.0
Repeated courses same drug	200	—
(All cases in above study: total recurrence rate 7.0%		
Newer antibiotics		
Aureomycin	35	60 (1951)
Terramycin	40	97.5 (Frye, 1952)
Neomycin	10	40 (1952)
Fumagillin	64	95.3 (McHardy, 1953)

* After McHardy *et al.* (modified) 1953.

offer promise when used alone in drug-refractive amebiasis such as we have encountered in the present study.

We are forced to the impression that the bacterial associates in the intestine in human amebiasis may play a more significant role in the pathogenicity of the disease than hitherto appreciated. However, the antibiotics now available do not offer very significant differences in percentage of patients cleared, over accepted synthetic amebicides of the nonantibiotic class. Weiser *et al.* (1953) have come to a similar conclusion. A critical review of the observations, and comparative findings of McHardy *et al.* (1953), while favoring fumagillin, provide approximately comparable clearance rates for several agents. These observations of McHardy *et al.* in comparison with other studies cited by these authors, reveal that for synthetic (non-antibiotic) amebicides the recurrence rate was 7.0 per cent (to 1945), whereas for the newer antibiotics used alone, fumagillin and terramycin had recurrence rates of 4.7 per cent and 2.5 per cent respectively. One would question the significance of these differences, which are summarized in Table 3.

While we have been able to take far greater precautions in attempting to prevent reinfection in the present study, one possible avenue of reinfection by contact with chronic cyst passers remained. The mentally deficient children included in this study have been known to drink out of toilet bowls which were supplied by water having a high bacterial count. The drinking water, however, was found to have a low bacterial count. This possibility does not explain the difference in response to the various treatment regimes tested.

SUMMARY

After suggestive studies in vitro and trials in 49 macaques, 52 episodes of amebic infection in mentally defective children were treated with erythromycin alone, with fumagillin alone, and with these antibiotics in combination. Two recurrences of 17 treated cases were noted after erythromycin therapy, 5 of 21 after the two antibiotics were given concurrently, and 6 of 14 after fumagillin was given alone. The final test regimes followed by the 13 human patients whose infection recurred, provided clearance for all but one of the original group of patients having *E. histolytica* infection.

ACKNOWLEDGEMENT

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ARSTHINOL (BALARSEN)[1]

A New Trivalent Arsenical for the Treatment of Intestinal Amebiasis and other Intestinal Protozoa

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Drugs containing arsenic have been used widely for many years as therapeutic agents in the treatment of colonic amebiasis. In general, these drugs have been moderately effective in mild to subacute cases of intestinal amebiasis, relatively inexpensive, and of a low order of toxicity.

During routine *in vitro* and *in vivo* studies of various agents potentially useful in the therapy of amebiasis of man, we have investigated an arsenical recently introduced for the treatment of yaws by Friedheim (1949). This compound designated by the latter as "STB" (N-2-acetylamino-4-methylol cyclo-(ethylenedimercoptoarseno)-phenol), now known as arsthinol (Balarsen), was administered by Friedheim to 88, and by Kivits (1951) to 244 patients with clinically active yaws in single oral doses of 10 to 20 mg./kg. daily for 5 days. Levy and Talley (1952) reported good results in 22 of 25 cases of amebiasis treated for 5 days with 10 mg./kg. a day of arsthinol. No signs of drug intolerance or intoxication were reported. The present communication deals with the use of this new arsenical as a therapeutic agent in human intestinal amebiasis.

MATERIALS AND METHODS

The patients treated were studied in the Tropical Disease Diagnostic Clinic (Lower East Side Health Center, N. Y. City Health Department), the medical clinic of the New York Regional Office of the Veterans Administration, and the Out-Patient Department and wards of Bellevue Hospital, New York. One patient was studied at the Beth Israel Hospital, New York. The diagnosis of amebiasis was established by the demonstration of cysts of *Endamoeba histolytica* in casually passed stool specimens (zinc sulfate centrifugal flotation) and/or trophozoites in postcathartic specimens passed directly in the laboratory, and when indicated, corroboration of species identification of amebae was obtained by culture and iron hematoxylin staining. In patients with moderately active disease in whom ulceration was observed proctoscopically, trophozoites of *E. histolytica* were demonstrated in material obtained by curettage and examined directly in saline and after iron hematoxylin staining.

A total of 167 adults and children with amebiasis of varying severity (princi-

[1] Balarsen (arsthinol, N. N. R.) was supplied for this study by Endo Products Inc., Richmond Hill, New York.

pally asymptomatic or only mildly so) were treated. Three fourths of the patients were Puerto Ricans and the remainder presumably acquired their infections abroad (Asia, Africa, Europe and South America). Treatment in all instances except when discontinued because of toxic symptoms or signs was in the form of tablets administered by mouth in single or divided doses daily for a total of 5 days. The patients of both sexes varied in age from 3 to 67 yr. and in weight from 12 to 85 kg. The daily dose varied from 4 to 22 mg./kg. (62.5 to 1137 mg. total).

RESULTS

The effect of treatment in terms of the disappearance of *E. histolytica* from the stools and toxic manifestations in relation to dosage of arsthinol are presented in Table 1. The definitiveness of apparent cures may be evaluated by the fact that 38 patients were followed for a month or less after treatment, 33 for 1 to 3 months, 15 for 3 to 6 months, 22 for 6 months to a year, and 58 for from 1 to 3 years after treatment. Approximately 50 per cent of the patients were observed for at least 6 months after therapy. During the follow-up period, an average of 5.7 stools per patient was examined.

There were a total of 20 treatment failures or a failure rate of 12 per cent. Of 50 patients who received less than 9 mg./kg. per day, there were 11 failures, or 22 per cent, whereas of 63 patients who received 9 to 10 mg/kg., the failure rate was only 5 per cent, suggesting that the lower doses were inadequate. On the other hand, there were 6 failures, or 11 per cent, in the group of 54 patients who received more than 10 mg./kg. This may mean that there is no correlation between therapeutic efficiency and doses above 10 mg./kg. In fact, 2 of the treatment failures in this group had previously experienced parasitic relapse after doses under 10 mg./kg. Conversely, one treatment failure after a dose of 20 mg./kg. was successfully re-treated with a dose of 8 mg./kg. and remained negative during a post-treatment observation period of 24 months.

There did not appear to be any correlation between the dose and the interval to parasitic relapse after treatment. Six patients had positive stools again within

TABLE 1

Results of treatment and incidence of toxicity

	BALARSEN DAILY DOSE mg./kg.					
	3–4	5–8	9–10	11–15	16–22	Total
Patients treated	7	43	63	24	30	167
Number of failures	2	9	3	2	4	20
Percentage of failures	33	21	5	8	13	12
Toxicity:						
Rash	0	0	3	1	2	6
G.I.	0	0	1	5	7	13
C.N.S.	0	0	0	0	2	2
Total	0	0	4	6	11	21
Percentage						12

a month, 9 within 6 months, and 5 after 6 months or more following treatment. Reinfection in Puerto Rican families must be considered a possible explanation for the long interval failures.

Effect of treatment on other parasites. Approximately 90 per cent of the patients treated with arsthinol had one or more helminthic or intestinal protozoan infections in addition to *E. histolytica*, when their amebiasis was treated. None of the helminths (*Ascaris, hookworm, Strongyloides, Trichiuris, Clonorchis* and *Schistosoma mansoni*) were eliminated as a result of arsthinol treatment of 114 patients who harbored one or more of these helminths. On the other hand, certain of the intestinal protozoa seemed to be definitively eradicated as a result of treatment. The number of patients positive for these parasites before and after treatment was as follows: *E. coli* 126 and 17, *Trichomonas hominis* 40 and 7, *Giardia lamblia* 28 and 11, and *Balantidium coli* 4 and 0. The effect on the other intestinal protozoa was not consistent or conclusive.

Clinical aspects. In the vast majority of the patients, symptoms or objective findings attributable to amebiasis were either mild or absent. In a small number of patients, however, amebic ulceration was noted during sigmoidoscopic examination, and in a few there were changes in the cecum visualized by barium x-ray studies which were compatible with lesions described in amebiasis. In these patients, the sigmoidoscopic and x-ray findings reverted to normal during the post-treatment observation period. In one patient with severe amebic dysentery, no improvement occurred during four days of arsthinol treatment after which additional therapeutic measures were instituted. No opportunity was therefore afforded in the present study to evaluate the efficiency of this drug in moderately severe or acute amebic dysentery. The therapeutic results reported must be considered then only in relation to the effect of arsthinol in eliminating *E. histolytica* from the stools of patients who have only minor or no symptoms.

Toxicity. A total of 21 patients, or 12 per cent of the entire group of 167 patients treated with arsthinol, had significant side reactions attributable to the drug. The principal symptoms or signs were related to the skin, gastro-intestinal tract, or central nervous system. Six patients developed generalized eruptions of the skin, usually on the fourth or fifth day of treatment, or in the following week. In one patient, the rash was maculopapular, in 3 it was morbilliform, and in 2 patients it was scarlatiniform. The rash was associated with fever (104°F.) in one patient and leucopenia (4530) and fever (102°F.) in another. In all instances, the rash lasted for from a few days to a week. The frequency of drug eruptions appears to be the same in the patients who received 9 to 10 mg./kg. per day as in those who received larger doses. No skin reactions were noted in 50 patients who received less than 9 mg./kg. per day.

Thirteen patients complained of violent abdominal cramps, vomiting, or diarrhea. These symptoms usually occurred between the second and fourth days of treatment, and subsided when the drug was discontinued. In 2 patients, the full five day course of treatment was not completed, because of vomiting and abdominal cramps. With one exception, all the significant gastrointestinal symptoms occurred in patients who received more than 10 mg./kg. of arsthinol a day.

The most serious toxic manifestations were those related to the central nervous system and occurred in 2 patients. One adult patient receiving 1.0 gm. of arsthinol daily (18 mg./kg.) became disoriented on the second day of treatment, and later experienced a convulsion. The patient was hospitalized in a semicomatose state, from which recovery took place within 48 hours. The second patient, a 6-year old child, became somnolent and stuporous on the fourth day of therapy (22 mg./kg.—500 mg. daily dose). The central nervous symptoms were associated with fever and leucopenia. The patient was hospitalized, treated with BAL, and completely recovered within 48 hours. The spinal fluid findings in both patients were not remarkable, and no causes other than possible drug reaction were found to explain the illnesses. Note is made of the relatively large doses of arsthinol these patients received.

It is evident that the administration of this arsenical is associated with toxic potentialities which may be serious especially if doses above 10 mg./kg. per day are given. With lower doses the incidence of drug reactions notably related to the skin or gastro-intestinal tract may be in the order of 6 per cent.

SUMMARY AND CONCLUSIONS

A new amebicidal drug, arsthinol, has been given to 167 patients with mild or asymptomatic amebiasis, and apparent definitive elimination of *E. histolytica* from the stools occurred in 88 per cent of the patients treated for 5 days with daily doses ranging from 3 to 22 mg./kg. Helminths are not affected by treatment with arsthinol but associated infections with *E. coli*, *Trichomonas hominis*, *Giardia lamblia* and *Balantidium coli* were eliminated from a significant per cent of patients harboring these protozoa.

Toxic manifestations related to the skin, gastro-intestinal tract or central nervous system were noted in 12 per cent of the patients treated with arsthinol, but 80 per cent of the reactions occurred with doses above 10 mg./kg. per day and half with doses of more than 15 mg./kg. per day.

Balarsen is a useful amebicidal agent for the treatment of mild or asymptomatic intestinal amebiasis and for certain other intestinal protozoa in safe dosages, but further experience is needed to ascertain whether the good results herein reported will be applicable to more severe or acute amebic dysentery.

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EPIDEMIOLOGICAL STUDY OF *ENDAMOEBA HISTOLYTICA* AND OTHER INTESTINAL PARASITES IN THE NEW HOPE COMMUNITY OF TENNESSEE

A Restudy After 21 Years

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Milam and Meleney (1931), who studied the prevalence of *Endamoeba hystolytica* and other intestinal parasites in the New Hope school district of Jackson County, Tennessee, in 1930, found that 38 per cent of the population showed *E. histolytica* cysts in fecal specimens. They made a careful study of environmental conditions in the community as related to the prevalence of *E. histolytica*. Later Frye and Meleney (1932) made additional environmental studies in the same community.

The present investigation concerned the same community and many of the same families studied by Milam and Meleney. Our purpose was to determine the present prevalence of *E. histolytica* and other parasites in the community; relate the findings to environmental conditions; and compare our findings with those of Milam and Meleney. The value of our comparison is enhanced by the fact that one of us (F. E. J.) did much of the laboratory work of the original study.

New Hope community consists of a group of small farms located in the rough highland rim country east of the Cumberland River in Jackson County, Tennessee. The present population, which is entirely of the white race, consists of 322 individuals divided into 79 households. There is an old but well maintained school in the community. Most adults are literate, but there were 31 households in which one of the parents had only third grade education or less. An intelligent interest in our project was manifested by most of the adult inhabitants.

The principal crops are corn and tobacco, the latter being the chief cash crop. Truck crops are produced and canned for houshold use by most of the families. The rough nature of the country prevents the use of modern farm machinery and restricts the size of farming operations.

The sanitary condition of the community is primitive by urban standards but quite good as compared with rural conditions elsewhere in the South. Only 18 of 79 households lacked privies and at least half the privies present were modern and well kept. Fecal contamination, seldom gross, was observed on the premises of 21 families; 55 had clean premises and no data were available for three. From observations made by four staff members, the families were classified as to their standards of cleanliness. Ten of the 79 families were classified as dirty but most were fairly clean (34 families) or clean (35 families). Eight of the residences were adequately fly-proofed, 47 inadequately screened, and 24 had no screens at all.

[1] Located at the Medical School of the University of Tennessee, 874 Union Avenue Memphis 3, Tennessee.

Flies were exceedingly abundant in most of the houses during the period of study. Wells were used as a source of water by 40 families and springs by 36 (no data on three families). Electric washing machines were possessed by 28 of the families and 36 had electricity. Other families washed clothes in tubs with water heated on wood stoves or in kettles in the yard except for a few with gas or hand machines. No family had piped water supplies.

Comparing our observations with those of Milam and Meleney 21 years earlier, several differences are noted. In 1930 only three privies were found in the community which then numbered 75 households; however, standards of cleanliness were not unlike present standards, as Milam and Meleney classified only 13 households as dirty (cf. our finding of 10). Since the original study the population decreased from 374 individuals to 322 but we found 79 houses as compared with 75 observed then. Many of the same families live still in the same houses studied by Milam and Meleney. A notable change in the economy was effected by the introduction since 1931 of tobacco as an important crop. Also since the original study the area has been opened to the outside by the construction of good hard surface roads. About the same proportion of houses are fly-proofed as observed by Milam and Meleney but a larger proportion now have inadequate fly-proofing. Finally, electricity has been brought into the community since 1931 but this is a recent happening and the full final effect may not yet be evident.

METHODS

The epidemiological methods used paralleled closely those used in a previous study by this laboratory of another Tennessee community (Eyles, Jones and Smith, 1953). In brief, each household in the New Hope community was visited after preliminary reconnaissance and mapping. Written notes were made on mimeographed forms and the following data were recorded: 1. A list of all the family members with sex, age, education, and occupation. 2. Observations of fecal pollution of premises. 3. Data on fecal disposal facilities. 4. Size and condition of house, with special attention to fly-proofing. 5. Observations on personal cleanliness of the individuals in the household. 6. Observations on the degree of cleanliness of the house, especially the kitchen. 7. Facilities for cleaning and laundry. 8. Type of water source.

In all, each family was observed upon at least four occasions and by at least four responsible staff members. The findings in which opinion played a part were decided after conference between the members participating in the study and were entirely independent of the parasitological findings.

The parasitological methods were identical to those followed in a previous study from this laboratory (Eyles, Jones and Smith, 1953). Three fecal specimens were examined from each individual included in the study, with the exception of three persons who furnished only two specimens each. The interval between collections was one week. After collection, specimens were immediately packed in portable ice boxes and shipped by railway express so that they reached the laboratory early on the following day.

Each specimen was examined directly in iodine solution, and in iodine solution after concentration by a modification of the zinc sulfate method. A sufficient number of *E. histolytica* cysts was measured with a filar micrometer eyepiece to determine the relative prevalence of large and small races, cysts measuring less than 10 micra being assigned to the latter.

FINDINGS

Prevalence of Endamoeba histolytica. Seventy-two persons were found to be infected with *E. histolytica* out of 322 examined, this amounting to 22.4 per cent of the population (Table 1). Thirty-nine or 49.4 per cent of the 79 families in the community had one or more individuals infected with *E. histolytica*. The community sample was 97 per cent complete. The age distribution of *E. histolytica* infection is shown in Figure 1. The percentage prevalence was very low in the 0- to 4-year age group, but reached its maximum level of nearly 35 per cent in the 5- to 9-year age group. Although declining, prevalence remained high until the oldest age group was reached. The low level (10 per cent) for the people 60 and over may have been a chance occurrence due to the small number of individuals studied.

Only small differences in sex prevalence were seen in the various age groups but these may have been due to chance as the overall prevalence in the two sexes was about the same. Both large and small races of *E. histolytica* were observed. Altogether 52 individuals had large race cysts only; 17 had small race cysts only; and 3 had both large and small race cysts.

Perhaps more interesting than the age and sex distribution is the distribution of infections by position in the household. This is summarized in Table 2. This tabulation emphasizes the fact that *E. histolytica* is most prevalent in the younger people of the New Hope community. It also shows that the adult female group, which presumably includes most of the people who prepare food, does not have significantly more carriers than the adult male group. As a matter of fact, 16 of

TABLE 1

Prevalence of intestinal parasites in the New Hope community

SPECIES	PERSONS			FAMILIES		
	Number	Number infected	Per cent infected	Number	Number infected	Per cent infected
Endamoeba histolytica	322	72	22.4	79	39	49.4
Endamoeba coli	322	138	42.8	79	61	77.2
Endolimax nana	322	156	48.4	79	67	84.8
Iodamoeba bütschlii	322	57	17.7	79	39	49.4
Giardia lamblia	322	34	10.6	79	19	24.1
Chilomastix mesnili	322	8	2.5	79	6	7.6
Ascaris lumbricoides	322	31	9.6	79	15	15.2
Necator americanus	322	6	1.9	79	5	6.3
Irichuris trichiura	322	3	0.9	79	3	3.8
Hymenolepis nana	322	2	0.6	79	2	2.5

the 39 *E. histolytica* families had no infection in a food handler; seven of these 16 families had infections in adult males only, eight had infections only in children, and one family had infections in both adult males and children. Of course, many of the 22 families in which a food handler was infected had infected adult males or infected children also.

Prevalence of other intestinal protozoa. The prevalence of other protozoa by individuals and by families is also given in Table 1. The proportions infected are such as would be expected in view of the high incidence of *E. histolytica* except that the prevalence of *Iodamoeba bütschlii* (18 per cent) is higher in proportion to that of *E. histolytica* than is reported in most studies.

Prevalences by age of host are shown in Figure 1. *Endamoeba coli* showed much the same pattern as *E. histolytica* having its highest prevalence in the 5- to 9-year age group. *Endolimax nana* and *I. bütschlii*, however, showed their highest prevalence in the 10- to 19-year age group. All species showed some decline after reaching the peak prevalence, but the prevalence of *E. coli* and *E. nana* did not fall markedly in the oldest age group. It is very interesting that the age distribution pattern of *E. histolytica* corresponds fairly closely in trend to that of *Giardia lamblia*. Prevalence by sex of host was not significantly different for the protozoa other than *E. histolytica* although slightly more females were parasitized.

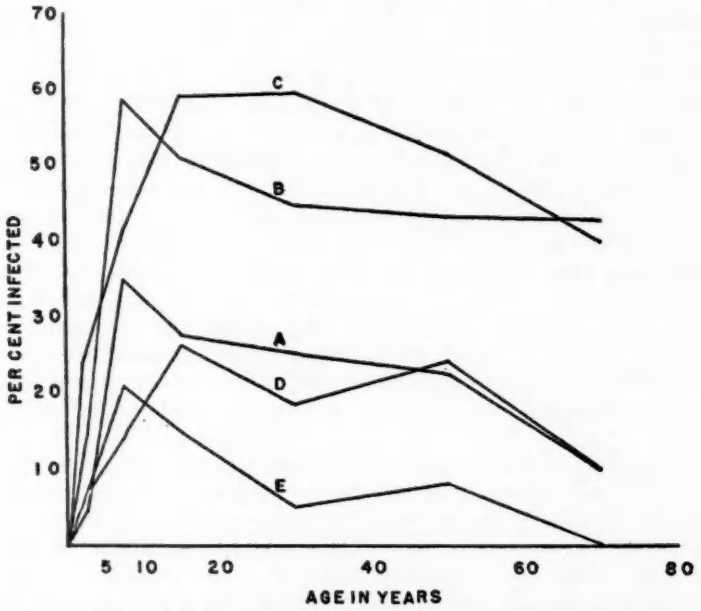

FIG. 1. Age prevalence of intestinal protozoa in the New Hope community. Curve A shows the distribution for *E. histolytica*, B for *E. coli*, C for *E. nana*, D for *I. bütschlii* and E for *G. lamblia*.

TABLE 2

Prevalence of E. histolytica *in relationship to position in the household*

POSITION IN HOUSEHOLD	NUMBER OF INDIVIDUALS	NUMBER POSITIVE	PER CENT POSITIVE
Adult males, mostly heads of households	79	16	20.3
Adult females, mostly heads of households	107	26	24.3
School-age children (6–16)	85	26	30.6
Pre-school children (0–5)	51	4	7.8

Prevalence of helminth parasites. The prevalence rates observed for the various helminth parasites are given in Table 1. The most frequent worm parasite was *Ascaris lumbricoides* which was found in almost 10 per cent of the population. Only scattered infections of worms other than *Ascaris* were detected.

Multiple parasite infections. Expected numbers of multiple infections, assuming strict independence of occurrence, were compared with observed numbers. As in the case of our earlier study (Eyles, Jones and Smith, 1953), multiple infections with amebic parasites occurred much more frequently than would be expected through the operation of chance alone. The occurrence of these parasites in conjunction with each other indicates that certain individuals are for some reason more subject to infection with amebae than others.

Contrasting with this, multiple infections of *G. lamblia* and of *Ascaris lumbricoides* and the intestinal amebae occurred no more frequently than would be expected. Factors which could be responsible for the observed association of the amebae are discussed in another paper (Eyles, Jones and Smith, 1953).

Correlation of parasitological findings and environmental conditions. Findings from the observations of the environmental conditions in and around the households were very similar to our findings in a rural West Tennessee community (Eyles, Jones and Smith, 1953). Clean families tended to have the lowest prevalence rates; where good fecal disposal facilities were present the families were least frequently infected. When no privy was present fewer families were infected than when an unsanitary privy was present. Premises with fecal pollution were more likely to be occupied by infected families, but the same was true to a much more marked degree for *Ascaris*. Prevalence rates were similar for families using wells as compared with springs. Although the above general associations were observed, the degree of association was very slight, as in the case of our earlier study.

Comparison with findings in 1930 in New Hope. Only limited comparisons can ordinarily be made between communities or groups studied for intestinal parasites due to variation in technic and other factors which might introduce bias. In the case of the two studies of New Hope community we believe limited comparison is safe since one of our investigators (F. E. J.) performed a large part of the technical work of the study reported by Milam and Meleney (1931). Admittedly, technical methods have improved through the years and our protocol provided for a more thorough parasitological followup than that of the earlier

TABLE 3

Comparison of prevalence rates of intestinal parasites in New Hope community at the present time and in 1930

	PRESENT STUDY			STUDY BY MILAM AND MELENEY IN 1930		
	Number examined	Number infected	Per cent infected	Number examined	Number infected	Per cent infected
Endamoeba histolytica	322	72	22.4	357	136	38.1
Endamoeba coli	322	138	42.9	357	250	70.0
Endolimax nana	322	156	48.4	357	141	39.5
Iodamoeba bütschlii	322	57	17.7	357	71	19.9
Giardia lamblia	322	34	10.6	357	68	19.0
Chilomastix mesnili	322	8	2.5	357	105	29.4
Ascaris lumbricoides	322	31	9.6	357	117	32.8
Necator americanus	322	6	1.9	357	21	5.9
Irichuris trichiura	322	3	0.9	357	38	10.6
Hymenolepis nana	322	2	0.6	357	5	1.4

study. These facts tend to emphasize rather than detract from the differences we found.

Overall prevalence of intestinal parasites, both protozoa and helminths, is definitely lower now than in 1930. Table 3 shows a comparison of the rates. In the present study we found only about three-fifths of the *E. histolytica* prevalence found in 1930. The rates for *E. coli* are similarly lower now. We found 48.4 per cent of the population with *E. nana* at present compared with 39.5 per cent reported by Milam and Meleney (1931). We do not believe this to represent a real increase. Most likely, the more advanced technical methods of the present

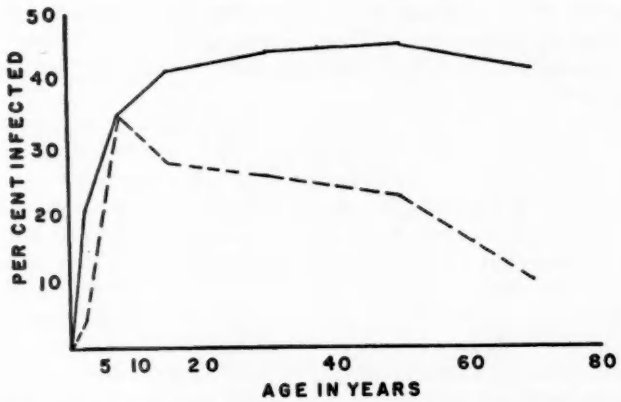

FIG. 2. Graph showing comparative age prevalence of intestinal protozoa in the New Hope community in 1930 and at the present time. Solid line gives age distribution found by Milam and Meleney in 1930; dotted line, that of present study.

study revealed a greater proportion of the infections with this species. *Chilomastix*, present in 29.5 per cent of the population in 1931, was found by us in only 2.5 per cent. *Giardia* has fallen from 19.0 to 10.6 per cent.

Examination of the comparative age prevalence distributions shows that in 1930 the highest prevalence of *E. histolytica* was in the 40- to 49-year age group (Fig. 2). Now the highest prevalence is in the 5- to 9-year age group. Present prevalence rates are almost the same as they were in 1930 in the youngest age groups. Most of the difference in prevalence between 1930 and 1951 can be accounted for by the fact that older children and adults now have lower parasitism rates.

Milam and Meleney give age distribution data only for *E. histolytica*. It is unfortunate that we cannot compare the other rates on the basis of age to see if the difference could be accounted for by a change in adult prevalence as in the case of *E. histolytica*.

Many of the persons examined by us in the older age groups were examined in the previous study. For instance the 20- to 39-year group of Milam and Meleney is the same (except for emigration and immigration) as our 40- to 59-year group. The rate for this group in 1930 was 44.3. It is now 22.6. *It would appear that half of these people have lost the* E. histolytica *infections they had 20 years earlier*. This loss must have been spontaneous for no large number of the population had been treated for amebiasis so far as we could determine.

Alternatively, the decrease in amebic prevalence following childhood could be explained on the basis of an acquired tolerance due to long-continued exposure to the parasites. This would assume the existence of undetected infections with low levels of cyst production. We believe that spontaneous cure rather than acquired tolerance is the proper explanation of the age prevalence curve. In the first place, in another study (Eyles *et al.*, unpublished observations) we have noted that the demonstrability of cysts in adults and in children is similar. Secondly, a curve similar to that of the present study should have been observed in 1931 if acquired tolerance were the explanation, for exposure to infection at that time must have been repeated.

Thus, it can be postulated that infections with *E. histolytica* tend to disappear spontaneously unless reinfection occurs. The change in the New Hope population is evidence of this and we have other unpublished evidence that such cures can be observed over the relatively short period of one year. It appears likely that some change has occurred in the New Hope community which interposes a barrier to the continued reinfection of the adult groups. We have summarized in the introduction some of the changes which have occurred during the period between studies. Our opinion is that the major change could be greater present day consciousness of the importance of avoiding fecal contamination. Certainly better fecal disposal facilities now exist. Cleanliness of house and person does not seem to have improved commensurately during the last 20 years; however, personal fastidiousness with regard to fecal contamination might not be reflected in the overall cleanliness picture.

Admittedly, other important changes have occurred in the community.

Electricity has been brought in recently, and tobacco was introduced as an important crop. We cannot estimate the effect of these factors on the parasitism rates. We can conclude, however, that the progressive changes in the community and the enlightenment which comes with such progress has resulted in marked reduction of parasitism.

Comparison with findings in West Tennessee. This laboratory has recently published findings of a similar epidemiological study in a rural community of Fayette County in West Tennessee (Eyles, Jones and Smith, 1953). We can compare this study with the present one as the parasitological protocols were identical. Our purpose in making the comparison is to find what differences exist and seek a rational explanation of the differences in the hope that light may be shed on the factors which determine the level of parasitism.

Table 4 presents prevalence rates in the two communities. The prevalence of all parasites was markedly lower in the West Tennessee community and this is in accord with the observation of Meleney *et al.* (1932) based on a state wide survey.

An important difference in the communities is that the West Tennessee population which we studied was mostly Negro, whereas, in New Hope the population was entirely white. We can find no authoritative work on comparative racial susceptibility, but in light of our findings in these two studies we are at present making such an investigation.

We sought an explanation of the lower West Tennessee rates in the comparative level of sanitation in the two communities, but it was the opinion of all of the persons concerned in these studies that general sanitation and cleanliness was much superior in the New Hope community where the higher rates were found. In other words, all of our environmental observations would have led us to suspect that the New Hope community would be less subject to parasitism.

An important difference in the two communities was the difference in terrain. New Hope community is in semimountainous country, whereas, the West Ten-

TABLE 4

Prevalence rates of intestinal parasites in New Hope community (present study) and Fayette County (Eyles, Jones and Smith, 1953)

	NEW HOPE COMMUNITY			FAYETTE COUNTY		
	Individuals examined	Number infected	Per cent infected	Individuals examined	Number infected	Per cent infected
E. histolytica	322	72	22.4	2657	278	10.5
E. coli	322	138	42.9	2657	590	22.2
E. nana	322	156	48.4	2657	733	29.1
I. bütschlii	322	57	17.7	2657	103	3.9
G. lamblia	322	34	10.6	2657	239	9.0
C. mesnili	322	8	2.5	2657	16	0.6
A. lumbricoides	322	31	9.6	2657	113	4.3
N. americanus	322	6	1.9	2657	4	0.2
T. trichiura	322	3	0.9	2657	1	0.04
H. nana	322	2	0.6	2657	21	0.8

nessee community was characterized by gently rolling topography. Associated with the mountainous topography was dense shade over much of the area. In the West Tennessee community a great portion of the country was open, particularly around the premises. We believe it is possible that the survival of cysts outside the host may be favored by the microclimatic conditions in the semi-mountainous New Hope community. The less extreme temperatures caused by the shade may favor the survival of cysts greatly. The desirability of further studies of the influence of external factors on the viability of cysts is indicated by this study.

Finally, a notable difference in the parasite picture in the two communities, for which we have no explanation, is the comparative frequency of the large and small races of *E. histolytica*. In West Tennessee we found the small race in over half of the persons infected with *E. histolytica*. In New Hope the large race was three times as frequent as the small. Since technical methods were identical we believe this represents a real difference. Actually the prevalence of the small race was about the same in both communities; the difference in overall prevalence being mostly accounted for by the much greater prevalence of the large race in New Hope.

In our report on intestinal parasites in a rural community of West Tennessee, we approached our data from the point of view of determining how the observations were or were not in accord with the various theories as to the mode of transmission of the intestinal amebae. All in all, the findings in New Hope, although based on less extensive data, follow the same pattern as those of the earlier study, and the data fit best the theory that transmission is by direct contact or contact with contaminated articles. The particular reasoning upon which this statement is based can be found in our earlier report (Eyles, Jones and Smith, 1953).

SUMMARY

A study of intestinal parasitism in the New Hope community of Jackson County, Tennessee, based on three fecal specimens examined from each individual, revealed the prevalence of *Endamoeba histolytica* to be 22.4 per cent. The prevalence of this parasite reached a maximum of 35 per cent in the 5- to 9-year age group and subsequently declined. Prevalence rates of the other intestinal protozoa were commensurately high, and a similar age distribution pattern was observed. *Ascaris lumbricoides* was the most frequent helminth parasite being found in 9.6 per cent of the population.

Compared with a previous study in 1930, amebae in general (except *E. nana*) are now much less prevalent. The difference in prevalence appears to be due primarily to a lower prevalence of parasitism in adults at the present time. The change in the age-prevalence relationship would indicate that spontaneous termination of parasitism is frequent.

Compared with a rural, negro population of West Tennessee, recently studied in a parallel investigation, the white New Hope community had much higher parasite prevalence rates, despite a higher level of sanitation and cleanliness.

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THE EFFECT OF COLD TEMPERATURES ON THE VIABILITY OF CYSTS OF *ENDAMOEBA HISTOLYTICA*[1]

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The marketing of food products has undergone many changes in recent years with the introduction of methods for the processing and distribution of foods in a frozen state. The question reasonably arises as to the transmissability of such infections as amebic dysentery by contamination of produce originating in the tropics and brought into this country for distribution.

Relatively little has been written concerning the survival of *E. histolytica* in a cold environment and those data which are available are insufficient to define what happens to the organism in a deep-freeze storage cabinet. Chang (1943), although working primarily with *E. histolytica* cyst suspensions at temperatures of 4° to 40°C., did make a few observations at −2° to −8°C. He found that at the lower temperatures cysts remained viable for only a few days, "presumably due to the physical effects of ice formation." Cysts at 4° to 5°C. lived thirty to forty days, and by implication and extension of his experimental cyst survival curve, this suggested that cysts maintained at 0°C. would remain viable for a period of eighty-seven days. This projected life span was decreased by one-third with every successive 10°C. rise in environmental temperature.

Weinman and McAllister (1947) subjected both cysts and trophozoites in twenty trials to freezing and thawing. In one instance, with culture material frozen at −15°C. and stored at the same temperature, growth occurred. They concluded that this phenomenon indicated not so much the inability of the organism to survive low temperatures as that the optimal conditions had not been achieved for survival.

Goldman and Johnson (1950) reported that freezing of stools was an unsatisfactory method for preserving cysts and eggs for subsequent demonstration by direct or zinc flotation technic. One may infer that these organisms deteriorate under such conditions.

Considerable attention has been given to the effects of higher temperature (4° to 68°C.) on the survival of *E. histolytica* (Wenyon and O'Connor, 1917; Penfold and Woodcock, 1916; Boeck, 1921, 1921-b; Yorke and Adams, 1926; Dobell, 1927, 1928; Wight and Wight, 1932; Tsuchiya, 1937; Chang and Fair, 1941). Many of these reports are based upon observations made at room and body temperatures. The effect of environmental influences on cysts was summarized by Andrews (1942). He reported that (a) drying and high temperatures are most unfavorable; (b) amebic cysts are more susceptible to desiccation than are bacterial spores; and (c) the best measurements of thermal tolerance indicate that the cooler the temperature, the longer the cysts can live.

[1] This project was supported in part by grants-in-aid from the Refrigerator Division, International Harvester Company.

The effect of freezing temperatures on other pathogenic parasites has also received little attention. Ransom (1916) and Augustine (1933) found proper refrigeration to be an effective means of destroying trichina cysts.

Faust (1947) subjected eggs of *Schistosoma japonicum* to a temperature of 4°C. over a four- and six-month period and found one and two per cent respectively still viable.

Weinman and McAllister (1947) investigated the problem of preserving endoparasites. They met with considerable success in preserving many of the parasites included in their study by subjecting the material to rapid freezing and storing at −70°C. to −76°C. Trypanosomes, leishmanias, Trichomonas and Plasmodium type organisms were successfully preserved by their methods.

In contrast to the relatively few reports on the effects of freezing temperatures on protozoan organisms, many reports exist as to freezing effects on bacteria and fungi (Tanner and Wallace, 1931; Straka and James, 1932; James, 1933; Smart, 1935; Lochhead and Jones, 1936; Weiser and Hargiss, 1946; Van Eseltine *et al.*, 1948). Such effects resulted in decrease or maintenance of microbic loads, but not in elimination of viable micro-organisms.

MATERIALS AND METHODS

A. Cultures containing trophozoites of the UC and NRS strains of *E. histolytica* were obtained from Dr. William Balamuth, Northwestern University.

1. The UC strain is one that had been isolated from a patient at the University of Chicago and grows with a multiple bacterial complex.

2. The NRS strain is one that had been isolated by Dobell from a monkey many years ago and is identical to the human strain in all respects. The NRS strain is also dependent upon a multiple bacterial association for growth.

B. Stock maintenance cultures incubated at 37°C. were prepared by interval transfers every forty-eight hours in Balamuth's medium (Balamuth, 1946).

C. Encystment of the UC and NRS strains was produced by Balamuth's technic (Balamuth, 1951).

D. Three International Harvester freezing chests (four cubic foot) were used, with Ramco differential and temperature controls for maintaining constant temperatures in the chests. A Westinghouse triple thermocouple unit with a graduated scale for determining temperatures in the chests was used for all temperature determinations. Thermocouples were placed inside each chest, attached to the bottom of the freezing chest baskets so as to be exactly in the center of the cooling area, and adjustments to the desired temperature made. One chest was constantly maintained at 4°C. (control temperature) through the two-year period of testing, while the other two were altered for the temperature range under study (0° to −35°C.). All temperatures were maintained within ±0.5°C.

E. The effect of cold temperatures ranging from 4°C. to −35°C. upon adequate cyst suspensions (5000–10,000/ml.) was investigated using two technics.

1) Cysts suspended in Ringer's solution were dispensed in 2 ml. lots into a series of test tubes 150 x 18 mm.; i.e., individual tubes were prepared for 4°C.

at 24 hour, 48 hour, 96 hour, 1 week, and 2 weeks incubation. Similar preparations were made for the other temperatures being investigated for any particular two-week period. In this manner a single tube could then be removed from each chest for any one time and temperature effect without disturbing cysts designated for later examination.

All tubes containing cysts to be observed at a particular temperature level were then placed in a common container, exactly in the center of the cooling area of the chests immediately over the thermocouple.

2) Two ml. of cysts suspended in Ringer's solution were inoculated into food packs containing a leafy vegetable (spinach) to imitate (though exaggerated by such mass inoculum) conditions of contaminated foods packed under conventional methods for distribution to consumers.

Several precautions were taken to prevent any possible accidents from occurring in the laboratory due to leaky packages or the cumbersome washing of the cysts from the leafy vegetable. One-pint paraffined cartons (4 x 3.5 x 2″) for frozen foods, lined with polyethylene plastic bags, were filled with blanched spinach leaves. Smaller plastic bags (3 x 2″) were then filled with blanched spinach leaves and seeded with 2 ml. from a prepared cyst suspension in Ringer's solution. These bags were then sealed by heat and planted into the middle of each of the cartons of spinach. This method afforded a rigid test of cold penetration to the center of the seeded implants as well as creating a safe and convenient method of handling cyst-contaminated material.

As with the cyst suspensions in test tubes, here again individual packs with time and temperature designations were placed into each of the freezing chests immediately over the thermocouples. Following proper incubation (24 hours to 2 weeks) under temperature conditions of 4°, 0°, −5°, −10°, −15°, −20°, −25°, −30° and −35°C., the individual implant from each pack was removed and thawed as rapidly as possible at 25°C. in individual sterile beakers. The contents were then removed, washed and centrifuged three times with sterile Ringer's solution and the sediment inoculated into excystment media.

F. In order to assure positive excystment cultures where viable cysts might still exist,[2] preconditioned[3] and enriched[4] media were simultaneously seeded from a split sample from any one of the time and temperature suspensions of cysts from the test tube set-up or implant technic. If no trophozoites were observed microscopically within four days in the initial excystment medium,[3, 4]

[2] In spite of the care taken, occasions arose where erratic results of the controls occurred. We feel that such occasions might arise due to the vagaries of the organisms themselves and media factors which cannot be controlled due to different stocks of egg yolk powder obtained throughout the year.

[3] Preconditioned Balamuth's medium: the oxidation-reduction potential of the medium is lowered by inoculating and incubating the medium at 37°C. with the prerequisite bacterial flora for each of the *E. histolytica* strains 24 hours before use. This medium was then made up to contain 2 per cent horse serum. A small amount of rice starch was added to each tube.

[4] Enriched medium: liver slant with Balamuth medium overlay containing 2 per cent horse serum and a small amount of rice starch.

subcultures were made into straight Balamuth's medium containing rice starch and again examined in 48, 72 and 96 hours for motile trophozoites. Only those tubes which then exhibited motile trophozoites were designated as positive excystment cultures.

G. Each test temperature run (to be referred to in tables as TTR) for the UC and NRS cyst strains included two temperature points in the range of 0°C. to −35°C. plus a control at 4°C., (4°C., N°C., N°C.), for a period up to 2 weeks. Consequently for each test temperature run, a fresh preparation of cysts was subjected to time and temperature effects followed by excystment attempts in vitro.

H. A trial run (4°C., N°C., N°C.) was gauged to be successfully completed by a positive excystment culture from cysts which had been maintained at 4°C. (control) for any specified period: viz., 24 hour, 48 hour, 96 hour, 1 week or 2 weeks. A trial run with a negative control for any specified period could not be assumed to be significant and so was eliminated from the present data to avoid confusion.[5]

RESULTS

Tables 1-A and 1-B show the time and temperature relationship to the number of positive excystment cultures.

Examination of these data indicates several phases in excystment under such conditions: first, *maintenance of viability* for cysts exposed to 4° to −5°C.; secondly, a *transitory phase* in which excystment is possible only through the first forty-eight hours at −10°C.; thirdly, a *phase of nonexcystment* when cysts are exposed to temperatures of −15°C. and lower for a minimum of twenty-four hours.

Figure 1 represents the results from Table 1-A and 1-B. Per cent positive excystment cultures for cysts over a two-week period of observation are based upon the total of successful excystment cultures obtained at the control temperature of 4°C. for each test temperature run.

The few results with spinach packs seeded with both strains of amebic cysts gave essentially the same viability spectrum as that obtained with cysts suspended in Ringer's solution and stored in test tubes at the appropriate temperature (Tables 2-A and 2-B).

Per cent positive excystment cultures for cysts maintained at N°C. in spinach food packs over a two week period of observation is represented in Figure 2. As in Figure 1, the total of successful excystment cultures obtained at the control temperature of 4°C. for each test temperature run is considered as 100 per cent.

[5] Sixty-eight trials with cysts (strain UC) suspended in Ringer's solution resulted in fifty-nine acceptable trial runs at various time and temperature designations. Fifteen trial runs with cysts (strain UC) seeded in spinach packs all proved successful. The results with strain NRS were somewhat less gratifying, as thirty-six out of sixty-eight trial runs proved to be successful with cysts suspended in Ringer's solution at various time and temperature designations, while thirteen out of fifteen trial runs with cysts (strain NRS) seeded in spinach packs were evaluated as acceptable.

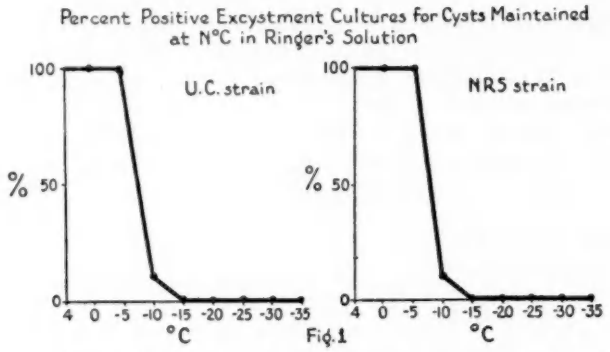

Fig. 1

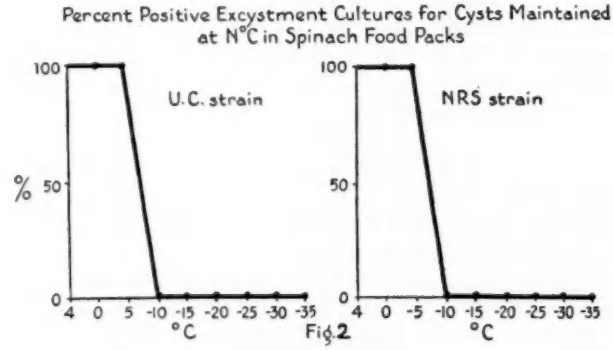

Fig. 2

FIGS. 1 and 2

No explanation as to the probable loss of viability has been undertaken, as the primary purpose of this investigation was to determine the effect of cold temperatures on the viability of cysts of *E. histolytica*. Such theories as ice formation within the organism disrupting the protoplasm, or improper methods of melting the frozen mass, and even improper methods of quick freezing must remain questions for further investigation.

TABLE 1-A

The effect of time and temperature on cysts suspended in Ringer's solution

TIME OF INCUBATION	NUMBER OF POSITIVE EXCYSTMENT CULTURES AT N°C.—UC strain															
	Trials	TTR #1			Trials	TTR #2			Trials	TTR #3			Trials	TTR #4		
		4°	0°	−5°		4°	−10°	−15°		4°	−20°	−25°		4°	−30°	−35°
24 hr.	4	4	4	4	5	5	2	0	3	3	0	0	3	3	0	0
48 hr.	4	4	4	4	4	4	1	0	2	2	0	0	1	1	0	0
96 hr.	2	2	2	2	4	4	0	0	2	2	0	0	1	1	0	0
1 wk.	4	4	4	4	3	3	0	0	3	3	0	0	3	3	0	0
2 wk.	4	4	4	4	2	2	0	0	2	2	0	0	3	3	0	0
Totals	18	18	18	18	18	18	3	0	12	12	0	0	11	11	0	0

TABLE 1-B

The effect of time and temperature on cysts suspended in Ringer's solution

TIME OF INCUBATION	NUMBER OF POSITIVE EXCYSTMENT CULTURES AT N°C.—NRS strain															
	Trials	TTR #1			Trials	TTR #2			Trials	TTR #3			Trials	TTR #4		
		4°	0°	−5°		4°	−10°	−15°		4°	−20°	−25°		4°	−30°	−35°
24 hr.	2	2	2	2	4	4	1	0	2	2	0	0	2	2	0	0
48 hr.	2	2	2	2	3	3	1	0	2	2	0	0	1	1	0	0
96 hr.	2	2	2	2	3	3	0	0	2	2	0	0	1	1	0	0
1 wk.	2	2	2	2	2	2	0	0	—	—	—	—	1	1	0	0
2 wk.	2	2	2	2	1	1	0	0	1	1	0	0	1	1	0	0
Totals	10	10	10	10	13	13	2	0	7	7	0	0	6	6	0	0

TABLE 2-A

The effect of time and temperature on cysts seeded into spinach food packs

TIME OF INCUBATION	NUMBER OF POSITIVE EXCYSTMENT CULTURES AT N°C.—UC strain															
	Trials	TTR #1			Trials	TTR #2			Trials	TTR #3			Trials	TTR #4		
		4°	0°	−5°		4°	−10°	−15°		4°	−20°	−25°		4°	−30°	−35°
24 hr.	2	2	2	2	1	1	0	0	1	1	0	0	1	1	0	0
1 wk.	2	2	2	2	1	1	0	0	1	1	0	0	1	1	0	0
2 wk.	2	2	2	2	1	1	0	0	1	1	0	0	1	1	0	0
Totals	6	6	6	6	3	3	0	0	3	3	0	0	3	3	0	0

TABLE 2-B

The effect of time and temperature on cysts seeded into spinach food packs

TIME OF INCUBATION	NUMBER OF POSITIVE EXCYSTMENT CULTURES AT N°C. NRS strain															
	Trials	TTR #1			Trials	TTR #2			Trials	TTR #3			Trials	TTR #4		
		4°	0°	−5°		4°	−10°	−15°		4°	−20°	−25°		4°	−30°	−35°
24 hr.	2	2	2	2	1	1	0	0	1	1	0	0	1	1	0	0
1 wk.	2	2	2	2	—	—	—	—	1	1	0	0	1	1	0	0
2 wk.	2	2	2	2	—	—	—	—	1	1	0	0	1	1	0	0
Totals	6	6	6	6	1	1	0	0	3	3	0	0	3	3	0	0

SUMMARY

In vitro observations demonstrate that (1) temperature levels of −15°C. or lower for twenty-four hours or longer are required to produce complete loss of viability of *E. histolytica* cysts; (2) significant reduction in viability is attained at temperature levels of −10°C.

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HUMAN EPIDEMIC IN COLOMBIA CAUSED BY THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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This paper presents the preliminary results of an investigation initiated in June, 1952, on occasion of a human outbreak characterized by a mild and short febrile disease observed in Espinal, Departamento del Tolima, Colombia, South America. A virus was isolated from two cases and very high titers of specific antibodies were found in the sera taken from 10 more persons during their convalescence. Studies which are still in progress indicate that the isolated agent is a strain of the Venezuelan equine encephalomyelitis virus.

The presence of the epizoötic form of Venezuelan encephalomyelitis has been previously recorded in Espinal as well as in other places of Colombia, where the disease is commonly known as "peste loca" (González, 1953). The possibility of human epidemics under natural conditions has been suspected before the actual outbreak (Albornoz, 1933 and Velasquez, 1939 cited by Soriano and Figueroa, 1942; Gallia and Kubes, 1944). As far as we know there are only two recorded natural infections in man (Randall and Mills, 1944; Gilyard, 1945), both of them being fatal cases. On the other hand, laboratory infections are by no means uncommon, indicating a high degree of infectiveness of the agent. (Casals *et al.*, 1943; Lennette and Koprowski, 1943; Gallia and Kubes, 1944; Koprowski and Cox, 1947).

EPIDEMIOLOGICAL FEATURES

Locality. Espinal is a village (74°, 53′, 35″ West of Greenwich; 4°, 09′ North of the Equator) situated in the upper Magdalena valley in the middle of a horse and cattle breeding country. The altitude is 438 meters (1440 feet) above sea level, with a mean temperature of 28°C. The population is about 9,000.

Incidence. Our knowledge of the epidemic course of the disease is very limited, since, as was stated above, we became engaged in the study of the problem only in June 1952, when the epidemic was reaching its end and we were able to study only two cases during the acute attack and 12 more persons who, it was said, had had the disease several weeks before. Since June no more cases have been observed, and the data previous to June are based entirely on the information given by the few local physicians. According to this information the first cases occurred in March 1952—not less than 70 cases were recorded from March to June—and the disease, which was recognized as different from those usually observed in the area, was tentatively diagnosed as a dengue-like fever. It is

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impossible to estimate even roughly how many human cases infected with the Espinal virus occurred because the local people consult doctors only rarely for mild and short illnesses and the extremely poor medical and public health facilities do not insure correct diagnoses, which are based only on clinical grounds. However the number of cases seems to have been rather high since the virus was isolated from both of the only two patients whom we were able to study during the acute phase of the disease and since we demonstrated very high titers of specific antibodies in 10 out of the 12 persons studied by us several weeks after the clinical attack (cases 3 to 12, Table 4). These titers can be explained only as elicited by a previous infection. It is quite probable therefore that the vast majority of the cases diagnosed by the local physicians as dengue-like fevers really were infections with Venezuelan equine encephalomyelitis virus. No differences in the age-incidence were observed.

Transmission. With regard to the possibility of the V.E.E. virus being transmitted by insects, it is worth while mentioning that during the epidemic, *Aedes aegypti* was found in 687 houses and *Culex fatigans* in 214 out of the 2295 houses of the village. In June the epidemic apparently was declining. During the last days of that month and the first weeks of July, the locality was thoroughly sprayed with D.D.T. The end of the outbreak coincided with this spraying.

It should be mentioned also that under laboratory conditions a few specimens of *A. aegypti* and *C. fatigans* which were allowed to feed on mice infected with the Espinal strain showed the presence of this agent 8 days after the infective meal, as demonstrated by inoculation of the ground mosquitoes into mice.

CLINICAL PICTURE

The clinical picture according to the collected data may be summarized as follows: sudden onset with malaise, chills and fever; nausea and/or vomiting; headache; muscle and bone pains. The fever lasted from 24 to 72 or 96 hours, when the temperature returned to normal quite suddenly. After the disease a period of 2–3 weeks of marked asthenia was observed. No other symptoms or complications have been recorded. A convulsive attack during the convalescence period, as well as some mental manifestations (loss of memory and changes in character), observed in a boy aged 8 years were attributed to the disease, but there is no certainty of an etiological relationship between these symptoms and the virus infection. The diagnosis in this case was established by means of the neutralization test (patient no. 4, Table 4). Apparently no fatal cases were observed.

CHARACTERISTICS OF THE VIRUS

Isolation. On June 15th, 1952, a woman (*L. de B.*) 38 years old and her daughter (*M. B.*) 3 years old, were bled in Espinal approximately 38 hours after the onset of the disease. The two samples were sent to Bogotá (Instituto Carlos Finlay) and were in transit for 36 hours without refrigeration. After 48 hours in the ice-box the sera were separated. With each serum a group of six 21-day-old albino-Swiss mice were inoculated intracerebrally. At the same time one of the

TABLE 1

Comparison of mortality in mice inoculated subcutaneously with filtered and unfiltered dilutions and intracerebrally with unfiltered dilutions of the Espinal virus

ROUTE OF INOCULATION	VIRUS DILUTION					TITER
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
S.C. (filtered)	6/6*	6/6	4/6	2/6	0/6	$10^{-7.5}$
S.C. (unfiltered)	6/6	6/6	6/6	0/6	0/6	$10^{-7.5}$
I.C. (unfiltered)	6/6	6/6	6/6	2/6	0/6	$10^{-7.7}$

* Mortality ratio.

authors inoculated himself subcutaneously with 0.5 ml. of a pool of the two sera (0.25 ml. of each serum). The mice developed a fatal infection with C.N.S. involvement, and the volunteer experienced a mild febrile disease. A filterable virus was recovered from the mice as well as from the volunteer's blood serum. This procedure enabled us to demonstrate the presence of a virus in both of the tested sera.

Filterability. The isolated virus is readily filterable and passes freely through E.K. Seitz filters (10 pounds pressure), as shown in Table 1.

EXPERIMENTAL INFECTIONS

Man. Twenty-four hours after the inoculation the volunteer experienced a chilling sensation lasting for two hours and the temperature reached 38.8°C. Intense backache, moderate insomnia and nausea were observed. The physical examination revealed nothing except low blood pressure (100/55 mm. Hg) and a mild pharyngitis. The blood showed leukopenia (2,300 with normal differential percentages). The fever and the acute stage lasted for 48 hours. Thereafter lassitude and asthenia were present for three weeks. The number of w.b.c. attained normal levels one week after the onset of the disease. At the site of inoculation neither local nor regional reaction was noticed. The patient was bled 41 hours after inoculation and the presence of circulating virus was demonstrated by blood serum inoculation into mice and defibrinated blood injected intraperitoneally into guinea pigs.

It should be added that in the course of the investigation two other persons directly engaged in the work developed a disease identical to that observed in the human volunteer. From both a virus was isolated and later, high titers of neutralizing antibodies were demonstrated in their sera specific to the Venezuelan and the Espinal viruses. From the studies already performed and since these persons were working simultaneously with both viruses, it is impossible at present to ascertain which one determined the disease.

Further studies will indicate whether other persons in our laboratory have contracted the infection in the course of routine work. The idea of vaccinating everybody in the Institute was discarded after we learned of the relatively high rate of virus infection produced by the vaccine itself (Smadel, 1953). The possibility of the occurrence of laboratory contaminations in experimental animals

was also considered. The guinea pigs and rabbits used in our experiments were carefully isolated. Mice were used in groups of six animals, each group being kept in a different cage. According to the results of the previous tests and the pilot experiments (which included about 3,000 mice) it was decided that isolation of the sick mice from those remaining in the same cage was not necessary. These tests as well as the work reported here showed that infections attributable to direct contamination from mouse to mouse never occurred.

Albino-Swiss mice, Rockefeller. This animal is very easily infected by any of the utilized routes (intracerebral, intraperitoneal, subcutaneous and intranasal). Mice die in all cases with typical symptoms of C.N.S. involvement, the virus being always present in the brain. No significant differences in susceptibility were observed when the same doses of infected mouse brain were injected comparatively by intracerebral and subcutaneous routes, as shown in Table 1. Death however, occurs at an earlier period of time after intracerebral injection. Animals 20–190 days old inoculated intracerebrally with 0.03 ml. of a 10 per cent suspension of infected mouse brain die 2–5 days later. With regard to age we have not observed differences in the susceptibility of these mice. The high degree of virulence for mice regardless either of the route of inoculation or the age of the animals has been previously recognized as peculiarly characteristic of the Venezuelan type of virus (Lennette and Koprowski, 1944).

Guinea pig. The infection is always fatal. High fever is observed as early as 12–24 hours after intraperitoneal injection, C.N.S. involvement is present, and the animal usually dies 48–96 hours after inoculation.

Rabbit. The course of infection in rabbits was irregular according to our very limited experience with this animal. In some cases the animal died with a picture similar to that observed in guinea pigs. In other instances the rabbits appeared resistant to large doses of virus (0.5 ml. of a 10 per cent suspension of infected mouse brain injected subcutaneously), without manifesting any symptoms of infection, although prior to inoculation they showed no neutralizing antibodies.

The Venezuelan equine encephalomyelitis virus (Kubes-Soriano strain) produced in mice, guinea pigs and rabbits an infection identical to that caused by the Espinal virus.

IDENTIFICATION OF THE VIRUS

On the basis of the behavior of the isolated virus in experimental animals it was suggested by Dr. M. Roca-García, of the Lederle Laboratories and by Dr. A. Lleras of the Colombian Public Health laboratories that we should compare the virus (which we will call the "Espinal virus") with the Venezuelan encephalomyelitis virus. The only available strain of the latter was a pool from two different sources: the original strain isolated in Venezuela by Kubes (Kubes and Rios, 1939) and the strain recovered from a horse in Bogotá, Colombia by Soriano (Soriano and Figueroa, 1942; Samper and Soriano, 1943) and identified by Kubes himself as the Venezuelan virus (Kubes, 1943). Cross immunity tests as well as neutralization tests with immune sera were performed with the Espinal and the V.E.E. viruses.

TABLE 2

Cross immunity tests in guinea pigs, employing the Venezuelan equine encephalomyelitis virus and the Espinal strain virus; subcutaneous inoculation

IMMUNIZING AGENT	CHALLENGING VIRUS	
	V.E.E. virus†	Espinal virus‡
V.E.E. virus		0/10*
Espinal virus	0/10	
Normal	6/6	6/6

* Mortality ratio.
† 14,000,000 mouse LD_{50} were used in the test.
‡ 40,000,000 mouse LD_{50} were used in the test.

Cross immunity tests. Two groups of mice and guinea pigs were immunized, one with V.E.E. virus and the other with Espinal virus under identical conditions. The animals received first brain-vaccines prepared according to the method described by Lennette and Koprowski (1944) and then very small amounts of the homologous living viruses. Nine days after the last immunizing dose of living virus, the cross tests were performed in guinea pigs as shown in Table 2 and in mice (Table 3). Each animal received subcutaneously 0.03 ml. of the respective virus dilution, except those included under dilution 10° which received 0.3 ml. of dilution 10^1. The results clearly indicate a complete cross protection.

Neutralization tests, human sera. Inactivated sera obtained from patients during the acute stage of the disease or during the convalescent period were tested simultaneously with tenfold serial dilutions of both viruses. The sera had been stored at 0–4°C. Equal amounts of serum and virus dilutions were mixed and incubated at 37°C. for one hour. Six mice (albino-Swiss) were inoculated with 0.03 ml. of each serum-virus mixture. The human sera were used without being diluted except in case of patient No. 2 (27th day), when a 1:2

TABLE 3

Cross immunity tests in mice, employing the Venezuelan equine encephalomyelitis virus and the Espinal strain virus; subcutaneous inoculation

IMMUNIZING AGENT	CHALLENGING VIRUS																			
	V.E.E. virus dilutions										Espinal virus dilutions									
	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
V.E.E. virus	0/4*	0/12	0/11								0/4	0/11	0/12							
Espinal virus	0/4	0/12	0/11								0/4	0/12	0/11							
Normal						6/6	6/6	6/6	2/6	1/6†						6/6	6/6	6/6	4/6	0/5‡

* Mortality ratio.
† Titer of challenge $10^{-7.9}$.
‡ Titer of challenge $10^{-8.3}$.

dilution of serum in 10 per cent normal human serum in saline was employed. The mixtures were inoculated subcutaneously except in the case of patient No. 1 (27th day) in whom the intracerebral route was used. As controls normal human inactivated serum and virus mixtures, under the same conditions as in the tests, were titrated. As source of virus, a 1:10 suspension prepared from brains of paralyzed mice infected intracerebrally with the respective virus was used. This suspension was considered as dilution 10^{-1}. The titers of the mixtures were computed according to the formula of Reed and Muench (1938). For simplification, neutralization indices of 1 to 50 were expressed as the nearest whole number, of 51 to 100 to the nearest 10, 101 to 1,000 to the nearest 100, 1,001 to 10,000 to the nearest 1000, and so on, as recommended by Olitsky and Casals (1947).

Sera from 12 persons were studied: in 7 cases, only one sample of serum was tested; in 3 cases, blood was taken both during the early stages of the disease and several weeks or months later. In two cases samples of serum were taken, the first two months and a second approximately seven months after the disease. The results, which are summarized in Table 4 indicate that all these people showed a high neutralization power against both V.E.E. and Espinal viruses.

TABLE 4

Neutralization tests in mice with human sera, using the Venezeulan equine encephalomyelitis virus and the Espinal strain virus; unless otherwise stated subcutaneous inoculation was employed

PATIENT NO.	DAYS SINCE FIRST SYMPTOMS	VENEZUELAN VIRUS			ESPINAL VIRUS		
		Log of titer of virus normal human serum mixtures	Log of titer of virus patient's serum mixtures	Log of LD_{50} (mouse) neutralized	Log of titer of virus normal human serum mixtures	Log of titer of virus patient's serum mixtures	Log of LD_{50} (mouse) neutralized
1	2*	−8.0	−8.0	0	−6.8	−6.7	0.1
	27	−6.6	−5.0	1.6‡	−6.0	<−2.0	>4.0‡
2	2†	−8.0	−8.0	0	−6.8	−6.6	0.2
	27	−8.0	−3.7	4.3§	−6.8	<−1.0	>5.8
3	4	−7.6	−6.8	0.8	−8.0	−6.5	1.5
	179	−7.6	−2.2	5.4	−8.0	−1.0	7.0
4	60	−7.6	−1.0	6.6	−8.0	<−1.0	>7.0
	201	−7.6	−3.0	4.6	−8.0	−1.9	6.1
5	61	−7.6	−1.9	5.7	−8.0	<−1.0	>7.0
	202	−7.6	−3.6	4.0	−8.0	−3.8	4.2
6	20	−8.1	<−1.0	>7.1	−6.7	<−1.0	>5.7
7	41	−7.6	−1.0	6.6	−8.0	<−1.0	>7.0
8	42	−7.6	−1.4	6.2	−8.0	<−1.0	>7.0
9	42	−7.6	<−1.1	>6.5	−8.0	<−1.0	>7.0
10	44	−7.6	<−1.1	>6.5	−8.0	<−1.0	>7.0
11	54	−7.6	−1.9	5.7	−8.0	<−1.0	>7.0
12	59	−7.6	−1.0	6.6	−8.0	<−1.0	>7.0

* Virus was isolated from this sample of serum (*L. de B.*).
† Virus was isolated from this sample of serum (*M. B.*).
‡ Intracerebral route employed.
§ Serum was diluted 1:2 in 10% normal human serum in saline.

In patients nos. 6, 9 and 10 it is impossible to compare the relative neutralizing power against each one of the two viruses, since no end point of both titrations was obtained. In the remaining sera, the neutralization index seems to be higher with the Espinal strain.

Neutralization tests, rabbit sera. One rabbit was hyperimmunized with V.E.E. virus and another with Espinal virus. Both animals received the same amount of infected brain suspension of the respective viruses on the same days, and were bled simultaneously to obtain serum which was stored at 0°C. Serial fourfold dilutions of each inactivated serum were prepared with normal inactivated rabbit serum, and each dilution was tested with tenfold serial dilutions of both viruses, in the same way as the neutralization tests with human sera were performed except that the mixtures were incubated at 37°C. for 30 minutes and then at 4°C. for 26 hours. In a first experiment both hyperimmune sera were tested simultaneously with Espinal virus. Two weeks later fresh dilutions of the sera were prepared and tested simultaneously with V.E.E. virus. The results are given in Table 5 and in Figures 1 and 2, which show that the responses of both hyperimmune sera are similar in a general way. It seems, however, that the Espinal serum has higher neutralization indices for the homologous strain. (Espinal virus).

Through the courtesy of Drs. Koprowski and Roca-García of the Lederle Laboratories, we received a vial of V.E.E. rabbit immune serum. This serum, diluted 1:4, when tested simultaneously with our V.E.E. and Espinal viruses

TABLE 5

Neutralization indices of different dilutions of hyperimmune V.E.E. serum and hyperimmune Espinal serum against V.E.E. and Espinal viruses

SERUM USED	DILUTION OF SERUM	V.E.E. VIRUS		ESPINAL VIRUS	
		Log of titer	Log of LD_{50} (mouse) neutralized	Log of titer	Log of LD_{50} (mouse) neutralized
Normal	Undiluted	−6.8		−7.0	
Hyperimmune V.E.E. serum	1:4	<−1.0	>5.8	<−1.0	>6.0
	1:16	−1.2	5.6	−1.5	5.5
	1:64	−4.4	2.4	−4.2	2.8
	1:256	−5.8	1.0	−4.6	2.4
	1:1024	−5.5	1.3	−4.9	2.1
	1:4096	−5.3	1.5	−5.8	1.2
	1:16384	−5.8	1.0	−5.0	2.0
Hyperimmune Espinal serum	1:4	<−1.0	>5.8	<−1.0	>6.0
	1:16	−3.0	3.8	−1.6	5.4
	1:64	−4.9	1.9	−4.2	2.8
	1:256	−5.6	1.2	−4.3	2.7
	1:1024	−5.5	1.3	−5.7	1.3
	1:4096	−6.5	0.3	−5.2	1.8
	1:16384	−6.5	0.3	−6.2	0.8

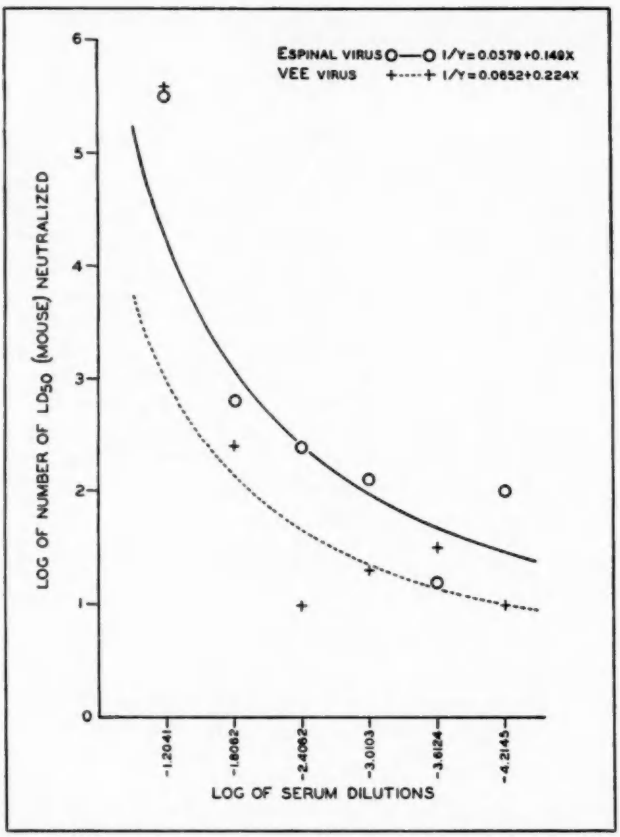

FIG. 1. Neutralization index of different dilutions of hyperimmune V.E.E. serum against Espinal and V.E.E. viruses.

(following the same technic used in our previous tests with rabbit sera), showed a neutralization index of $10^{5.3}$ with V.E.E. virus, and of $10^{2.5}$ with Espinal virus This fact is in contrast with the response of the V.E.E. rabbit serum prepared by us which showed comparatively similar neutralization indices with both viruses.

Comments. The cross immunity tests as well as the neutralization tests with both human convalescent sera and hyperimmune rabbit sera clearly show that the Espinal virus is very closely related, if not identical to the Kubes-Soriano strain of the Venezuelan equine encephalomyelitis virus with which the comparison has been made. The results of the cross immunity tests are definite in favor of the complete identity of both viruses. Those of the neutralization tests, although on the whole presenting a similar pattern, are not so obvious. From

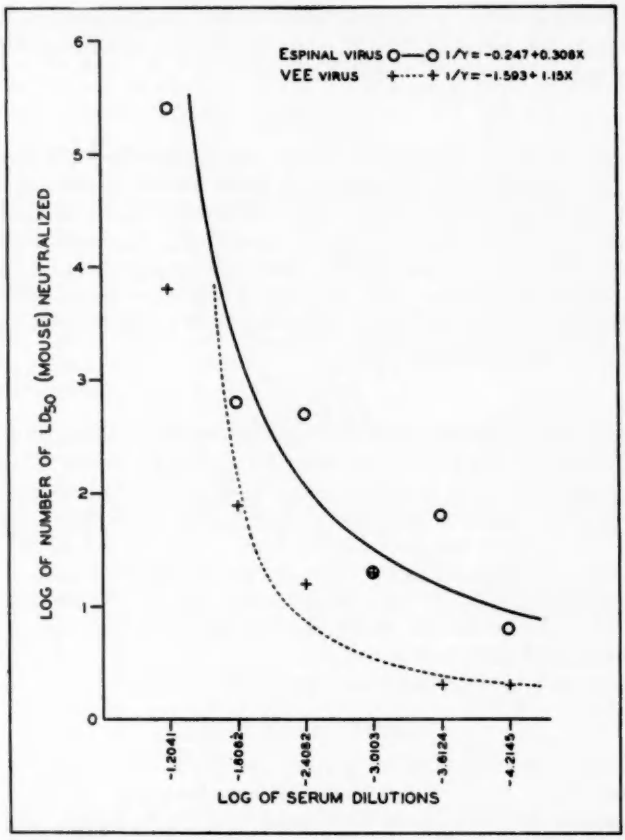

FIG. 2. Neutralization index of different dilutions of hyperimmune Espinal serum against V.E.E. and Espinal viruses.

the available data, the interpretation of the observed differences is difficult. These differences in the neutralization indices might be explained by chance variation, by the technical limitations of the test and/or by minor antigenic differences not demonstrable by the cross immunity tests. Certainly most of the differences shown by the convalescent sera could be considered as not significant. But others, such as those of Patients no. 4 (201st day) and no. 11, and those of Espinal immune rabbit serum, incline us not to disregard the possibility of minor antigenic differences between the viruses. The results given by the serum sent by Dr. Koprowski are in accordance with this line of thought. In the literature at our disposal regarding the possibility of antigenic differences in strains of the V.E.E. there are only the few data referred by Kubes (1943).

From the data presented we are inclined to believe that the Espinal virus is a

strain of the V.E.E. virus having possibly minor antigenic differences from the Soriano-Kubes strain of V.E.E. virus, differences which are not demonstrable by means of cross immunity tests.

DISCUSSION

The results of the study reported in this paper prove that the V.E.E. virus is capable of originating human epidemics under natural conditions. This fact is of importance for all those places where the epizoötic form of the disease is observed. Several authors have discussed the possibility that some of the obscure febrile diseases observed in the tropics, often diagnosed on clinical grounds as "grippe," "atypical malaria," etc., may really represent virus infections. The patients observed in Espinal clearly show that such a possibility has become a proved fact in this particular case.

SUMMARY

The outbreak in a human population of a disease characterized by a mild and short febrile entity was observed in Espinal, Colombia, South America from March to June, 1952. From 2 patients observed during their acute attack a virus was isolated. This agent when compared with a pool of the Kubes and Soriano strains of V.E.E. virus, has been identified as a strain of V.E.E. virus having possibly minor antigenic differences from the above mentioned pool, differences not demonstrable by means of the cross immunity tests. Furthermore, in 10 more sera from persons bled several weeks after the clinical attack, very high titers of neutralizing antibodies against both the pool of the Kubes and Soriano strains and the strain isolated in Espinal were demonstrated.

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THE INCIDENCE OF *SHIGELLA* ORGANISMS IN A GROUP OF EGYPTIAN VILLAGE CHILDREN[1]

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The endemicity of bacillary dysentery in Egypt has been known since early in the century when *Shigella* organisms were first isolated in this country (Ruffer and Wilmore, 1909, 1910; Wilmore and Savage, 1913). Studies on the etiology and epidemiology of the disease were stimulated by dysentery outbreaks among Allied troops during the first World War (Thomson and Mackie, 1917; Martin and Williams, 1917; Thomson and Hirst, 1918; Cowan and Miller, 1918; Manson-Bahr, 1919; Mackie, 1919; Drew and Fairley, 1920). During the next two decades a considerable amount of information was accumulated by local investigators. The seasonal incidence of the disease, the morbidity and mortality rates among Egyptian children, the occurrence of various *Shigella* species, the relationship with the fly breeding season, probable means of spread and other epidemiological aspects have been reported by Perry, 1928, 1930; Perry and Bensted, 1928, 1929; Shawki, 1928; Bensted, 1928; Khalid, 1928; Khouri, 1929; Biggam, 1930; Ali, 1935, 1940; Sabri *et al.*, 1937; Gohar, 1938; El Gholmy, 1941 and Korolos, 1942. The second World War further stimulated investigations in this field in Egypt, particularly in respect to the etiology and treatment of the disease in foreign military personnel (Wilson, 1942; Bulmer and Priest, 1943; Fairley and Boyd, 1943; Hone *et al.*, 1943; Wirts and Tallant, 1944; Heinemann, 1945; and Boyd, 1946).

Despite this volume of literature, the knowledge of the disease in Egypt is incomplete in many respects. The bulk of the information obtained during war times was from non-Egyptian military personnel living under entirely different conditions from the local population. Many of the early bacteriological data, derived without the more refined technics and materials of the present day, are difficult to interpret and apply now. More recent reports, based mainly on information from studies on hospital patients or from specimens submitted to public health laboratories, are limited in scope.

In planning long range epidemiological studies on gastroenteritis in Egypt it was felt that it would be advantageous to bring much of these basic data up to date. The present report is concerned with the incidence of *Shigella* organisms in a group of Egyptian children as determined by weekly rectal swab cultures during the period of one year.

MATERIALS AND METHODS

One hundred and sixteen children, between one week and three years of age, were obtained for this study from three adjacent villages (Sindbis, Quaranfil

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the U. S. Navy Department or the U. S. Naval Service at large.

and El Baradaa) 30 kilometers north of Cairo. Children were chosen in preference to adults because of availability and the greater frequency and severity of bacillary dysentery in the young. Village children were considered more representative than those in cities, since the majority of the Egyptian population lives in rural areas.

It is to be emphasized that this investigation did not include correlated clinical studies on dysentery among the children. However, by the use of certain criteria discussed below, it was possible to estimate the incidence of the disease in the group under study from the bacteriological data.

The children were from families of comparable social and economic standing. The living conditions of these villages have been well described by Weir *et al.* (1952). Village houses were constructed of mud brick, were poorly lighted and ventilated and were usually shared with the domestic animals. Bathing and washing facilities were inadequate and sewage and garbage disposal facilities were poor. Water was obtained from highly polluted canals or from wells often subjected to contamination. Cooking facilities were primitive and food was not protected from insects or dust. Human feces were deposited indiscriminately in any area that was convenient. Flies bred in tremendous numbers in human and animal wastes. Enteric and diarrheal disease rates were high and between 4 and 6 per cent of the population were enteric carriers.

Approximately one half of the subjects were children known to have had a recent history of diarrhea. The other half were selected at random from birth registration lists which provided no clinical information. Due to deaths, changes in residence or noncooperation of the mothers, the original 116 was reduced to 75. These 75 children were followed bacteriologically, via weekly rectal swab cultures, during the period between October 1951 and October 1952. Specimens were obtained by technicians during house visits. The swabs, carried in sterile tubes containing a few milliliters of glycerine and saline, were inserted a centimeter or two past the anal sphincter, rotated and then removed and replaced in the tubes.

At the laboratory Shigella-Salmonella agar (Difco) and MacConkey agar (Difco) plates were streaked with each swab. After incubation for 24 hours at 37°C. the plates were examined and suspicious colonies transferred to triple-sugar-iron agar (Difco) slants. These were incubated and then examined for reactions typical of *Shigella*. Those organisms showing such reactions were tested for their biochemical properties. Strains producing characteristic biochemical behavior were finally identified with *Shigella* group and type specific sera.

RESULTS

During the year in which the children were followed, 3,618 (93.8%) of a possible 3,855 weekly rectal swab specimens were obtained. *Shigella* organisms were recovered from 291 (8.04%) of the specimens. Two *Shigella* types were cultured from 4 of the positive swabs while each of the other 287 yielded a single type, making a total of 295 strains isolated.

Positive cultures were obtained from 73 (97.3%) of the 75 children. The

cumulative per cent of children from whom *Shigella* organisms were initially isolated, plotted against time, is shown in Fig. 1. From late October until December, 15 per cent of the children had positive cultures. During the winter months the rate of increase was almost static. From April until July the percentage climbed precipitously until over 80 per cent had yielded positive cultures. During late summer and early fall *Shigella* were isolated from all but two of the remaining children.

In Table 1 is shown the numbers of positive cultures recovered from each of the 73 children. The largest number of positive cultures from a single child was 15. The average number for the group was 3.98.

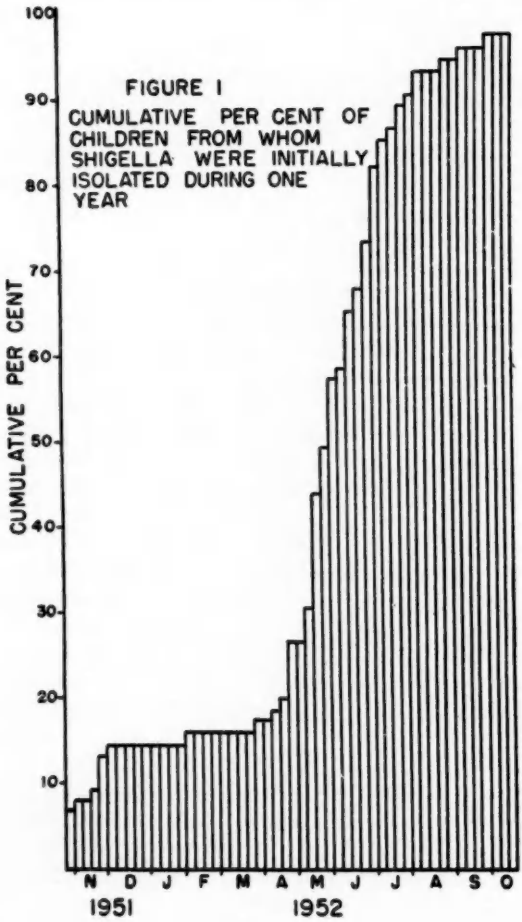

FIG. 1.

TABLE 1

Number of positive Shigella *cultures from individual children*

NUMBER POSITIVE CULTURES	NUMBER CHILDREN	TOTAL POSITIVE CULTURES
1	11	11
2	11	22
3	18	54
4	7	28
5	9	45
6	6	36
7	6	42
8	2	16
9	1	9
13	1	13
15	1	15
Total	73	291
Average		3.98

TABLE 2

Shigella *species and type distribution*

ORGANISM	NO. TIMES ISOLATED	PER CENT OF TOTAL
Group A:		
S. dysenteriae 1	5	1.7
S. dysenteriae 2	12	4.1
S. dysenteriae 3–7	13	4.4
	30	10.2
Group B:		
S. flexneri 1a	7	2.4
S. flexneri 2a	62	21.0
S. flexneri 3	60	20.3
S. flexneri 4a	30	10.2
S. flexneri 5	37	12.5
S. flexneri 6	6	2.0
S. flexneri Var "X"	7	2.4
	209	70.8
Group C:		
S. boydii 1	6	2.0
S. boydii 3	1	0.3
S. boydii 5	2	0.7
S. boydii 6	1	0.3
	10	3.4
Group D:		
S. sonnei	46	15.6
	295	99.9

TABLE 3

Number and per cent of children harboring various Shigella *species and types*

ORGANISMS	NO. CHILDREN	PER CENT OF TOTAL (75)
S. dysenteriae 1	4	5.3
S. dysenteriae 2	10	13.3
S. dysenteriae 3–7	11	14.6
S. flexneri 1a	5	6.6
S. flexneri 2a	28	37.3
S. flexneri 3	32	42.6
S. flexneri 4a	21	28.0
S. flexneri 5	15	20.0
S. flexneri 6	3	4.0
S. flexneri Var "X"	6	8.0
S. boydii 1	4	5.3
S. boydii 3	1	1.3
S. boydii 5	2	2.6
S. boydii 6	1	1.3
S. sonnei	27	36.0

TABLE 4

Alternation in Shigella *types found in replicate weekly rectal swab cultures*

NO. SHIGELLA TYPES	1	2	3	4	5	6	TOTAL
No. Children	21	26	10	13	2	1	73
Percent of 73 Children	28.7	35.6	13.7	17.8	2.7	1.4	100

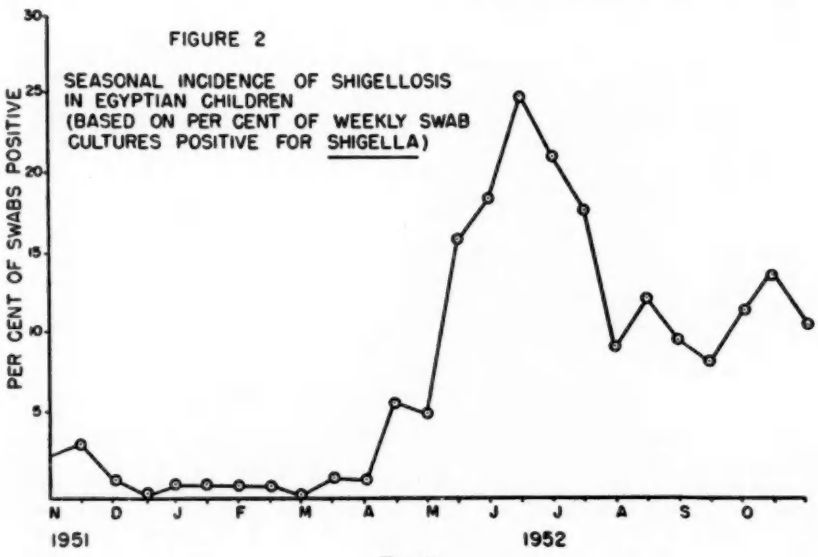

FIG. 2

Table 2 shows the *Shigella* species and type distribution among the 295 strains recovered. Group B *Shigella* strains accounted for 70.8 per cent of those isolated, 15.6 per cent were in group D, 10.2 per cent were group A strains and 3.4 per cent were group C organisms. *S. flexneri 2a, 3, 4a, 5* and *S. sonnei* were the types most commonly encountered.

In Table 3 are listed the numbers and percentage of children harboring the various species and types of *Shigella*. *S. flexneri 3* was recovered at least once from 42.6 per cent of the children, *S. flexneri 2a* from 37.3 per cent, *S. sonnei* from 36 per cent, *S. flexneri 4a* from 28 per cent and *S. flexneri 5* from 20 per cent. *S. dysenteriae 1* was found in only 5.3 per cent of the subjects.

Alternations in the species and type of *Shigella* found in individual children were frequently observed. As stated earlier, at least one positive culture was recovered from 73 (97.3%) of the subjects. More than one *Shigella* type was recovered from 52 individuals during the year. Twenty-six (35.6%) of the 73 children who had positive cultures harbored 2 types, 10 (13.7%) yielded 3 types, 4 types were recovered from 13 (17.8%), 2 children had 5 types and 6 *Shigella* types were recovered from one child. Table 4 illustrates the observed alternations.

The seasonal incidence of shigellosis in this group of Egyptian children, based on the percentage of positive weekly rectal swab cultures, is shown in Fig. 2. The incidence was low during the winter months, began to rise during the spring and reached a peak in early summer. After a decline during the hot summer period the incidence rose again slightly in the fall and subsequently fell to the level observed in the previous year.

DISCUSSION

This study was designed to obtain certain fundamental data for use in planning further epidemiological and clinical studies on gastroenteritis that are now in progress. Although no correlated clinical observations were made on the children during the times they were harboring *Shigella* organisms, it is possible to estimate the number of cases of bacillary dysentery that occurred among the group. If it be assumed that a clinical attack of shigellosis had occurred with each initial isolation of *Shigella*, or when a different species or type was isolated no sooner than one month following the previous attack, or when the same type or species was recovered after a three month interval of negative cultures, then there occurred in these 75 children 188 separate clinical cases of shigellosis, or an average of 2.5 cases per child per year.

Earlier investigators (Shawki, 1928; Perry and Bensted, 1928, 1929; Khalid, 1928; Bensted, 1928; Biggam, 1930; Ali, 1933; 1934, 1935, 1938; Sabri *et al.*, 1937; and Gohar, 1938) have differed widely in reporting the occurrence of various species and types of *Shigella* in Egypt. It has been usually agreed that *S. flexneri* was the principal etiological agent of bacillary dysentery but estimates of the occurrence of this species have ranged from 24 to 70 per cent. The results of the present investigation indicate an even greater prevalence of *S. flexneri* types, since, under the former system of classification, the 3.4 per cent of group

C organisms would be added to the 70.8 per cent of group B for a total of 74.2 per cent.

There have been few reports on the incidence of types of *S. flexneri* isolated in Egypt. Usually these have been concerned with strains recovered from foreign military personnel (Fairley and Boyd, 1943; Heinemann, 1945; Boyd, 1946). The preponderance of *S. flexneri 2a, 3, 4a* and *5* reported here does not compare very closely with the incidence found in British or American troops stationed in Egypt between 1940–45. Among the former *S. flexneri 2a* predominated, *S. flexneri 6* was common and *S. flexneri 1a, 3, 4a* and *5* were much rarer. In the American troops, *S. flexneri 1, 2a, 2b, 3, Var "X"* and *Var "Y"* comprised 63.5 per cent of the *S. flexneri* strains isolated. *S. flexneri 6* was common while *S. flexneri 4a* and *5* were rare.

There is also no close similarity between the incidence of other *Shigella* species reported here and that of the earlier investigators. In comparison with the present finding of a 1.7 per cent incidence of *S. dysenteriae 1*, the majority of 18 earlier investigators reported an incidence of 10 to 25 per cent of this species among Egyptian subjects. *S. dysenteriae 2* was either not isolated at all or was found to range from 0.7 per cent to 9.1 per cent as compared to the 4.1 per cent of this report. Similarly, *S. sonnei* was either not found or ranged between 0.5 and 40 per cent as contrasted with 15.6 per cent reported here.

It is not surprising that discrepancies exist between the *Shigella* species and type incidence presently reported and that found earlier. As pointed out by Weil (1947), the incidence of types varies even in the same locality from one time to another. In addition, since most of the previous reports were concerned with *Shigella* strains isolated from hospital patients, a heavy incidence of the more virulent types, such as *S. dysenteriae 1*, would be expected. Furthermore, few of the earlier studies extended over more than a few weeks or months. Species incidence, determined by surveys of short duration, may be unduly influenced by outbreaks from a single type or species. The time of the year that short surveys are made may also influence the results. It was noted during this study that most of the *S. sonnei* strains were recovered during the summer months and all of the *S. dysenteriae 1* strains were isolated in the fall. Had the study only covered either of these periods the distribution found would have been different. Of perhaps equal importance to the above factors was the use in the present study of media of a more selective nature, improved isolation technics and more sensitive typing sera. None of these were available 10 to 20 years ago.

The determination of the numbers of children harboring the various types of *Shigella* organisms was of value in planning an immunization program, now in progress, against shigellosis in a similar group of children. It was felt that the figures of Table 4 gave a more accurate picture of the etiological role of various types of *Shigella* than the data of Table 3, since there was no constancy in the number of positive cultures that were recovered from single individuals.

The seasonal incidence of shigellosis, as determined by positive cultures in this study, was comparable with that reported by other investigators. It was of interest to note again the close correlation between this incidence and the pre-

viously determined seasonal prevalence of houseflies in Egypt. It has been shown that the number of flies increase markedly from March through June, decline during the extreme heat of July and August and then increase again to a lesser extent in September and October. Such close correlation lends further circumstantial evidence to the probability that much of the bacillary dysentery in this area is fly borne. It was also of interest to note that during the height of the season as many as 25 per cent of the weekly swab cultures were positive for *Shigella*. This rate is comparable to that found during clinically defined, large scale epidemics of bacillary dysentery in less highly endemic areas.

SUMMARY

Seventy-five Egyptian village children were followed bacteriologically, via weekly rectal swab cultures, for one year for the presence of *Shigella* organisms. *Shigella* were recovered at least once from 73 (97.3%) of the subjects. On the average, each child had approximately four positive cultures during the year.

Two hundred and ninety-five strains of *Shigella* were recovered. Thirty (10.2%) were group A *Shigella*, 209 (70.8%) were group B, 19 (3.4%) were group C and 46 (15.6%) were group D. *S. flexneri 2a*, *3*, *4a*, *5* and *S. sonnei* were the most commonly encountered types. *S. dysenteriae 1* was rarely found. *S. flexneri 3* was recovered from 42.6 per cent of the children, *S. flexneri 2a* from 37.3 per cent, *S. sonnei* from 36 per cent, *S. flexneri 4a* from 28 per cent and *S. flexneri 5* from 20 per cent.

Alternation in *Shigella* types found in replicate weekly specimens from individual children was frequently observed. A distinct seasonal incidence in the recovery of positive *Shigella* cultures was noted.

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MYCETOMA OF THE HAND AND ARM CAUSED BY *MADURELLA*

Report of a Case with a Review of the Literature

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Mycetoma is a chronic, granulomatous process characterized by variously sized enlargements in the cutaneous surface which eventually give rise to intercommunicating sinuses and fistulas from which may be obtained variously colored granules comprising fungus elements of several genera and numerous species. The disease is usually localized to the extremities, chiefly the feet, and is invariably the result of traumatic inoculation.

Because the foot is the most common site, the disease was named "Madura foot" by Colebrook in 1846 (quoted by Castellani and Chalmers, 1919) on the basis of the work done by Gill of Madura, India, in the region where the disease was prevalent. The foot is not the only area involved since the infectious process may attack the leg, region of the knee, thigh, buttock and less commonly in chest, back, arm, hand, perineal region and the head. Accordingly, the name "Madura foot" is not well chosen and should be replaced by the more acceptable term mycetoma, created by Carter (1862), meaning a fungus tumor.

Mycetoma may be caused by numerous organisms including actinomycetes (*Actinomyces* and *Nocardia*); Ascomycetes (*Allescheria*, *Aspergillus*, *Penicillium*, *Sterigmatocystis*); Fungi Imperfecti (*Madurella*, *Indiella*, *Monosporium*, *Phialophora*, *Cephalosporium* or *Hyalopus*, *Glenospora* and others).

Chalmers and Archibald (1916) used the term maduromycosis, as caused by *Madurella*, to distinguish the lesion from those caused by actinomycetes. There is some justification for such a differentiation. Although the lesions may be identical clinically, they differ in that in actinomycosis or nocardiosis they may be easily transmitted from one area to another, being in many instances systemic in their spread. The lesions of maduromycosis, caused by *Madurella*, remain fairly well localized, invariably to the sites of trauma, and spread usually only by contiguity. Maduromycosis is considered to be a deep dermatomycosis with no evidence of systemic spread. From the histologic standpoint also there is a definite distinction of one from the other.

On the other hand, to adopt the term maduromycosis would mean that for each genus causing the disease, for which there is no existing terminology, a new term would have to be created. Such an increase in terminology would lend more confusion to an already complicated subject. In view of this it would seem best to adhere to the term mycetoma and refer to the lesion as mycetoma caused by the involved organism.

In this paper there is presented a report of mycetoma involving the hand and arm which is not a common observation. The organism causing the lesion was

identified as similar, in many respects, to *Madurella ikedai* Gammel, which may be synonymous with *M. americana* and possibly *M. mycetomi*. Actually, this represents probably the fourth case caused by *Madurella* involving the hand and/or arm and the first in a native born American.

REPORT OF A CASE

V. McG. (#201977), a 64 year old colored man, a cotton picker, who spent all his life on farms in Mississippi and Missouri, was referred to the Barnes Hospital with the chief complaint of a swollen and painful right arm.

Present illness

Fifteen years previously he had noticed a small (2.5 cm.) non-tender, freely movable, smooth nodule in the skin over the outer surface of the right wrist. The swelling and pain subsided and reappeared over a period of seven years. He did not recall any trauma to the area, fever, or foci of infection. This area became progressively larger and after seven years the "nodule" burst and hard black "birdshot" material exuded. The right forearm became swollen and tender but without fever, chills, axillary tenderness or joint stiffness. A few days later he noted small (1 cm.) tender sores around the wrist which exuded pus and occasionally black granules. The number of exudative areas gradually increased over the next few years to involve the entire forearm. For the next two years the swollen right forearm had a dull aching pain and gave him much discomfort.

For the past one year the right forearm and hand had felt extremely hot to the touch and there was stiffening of the elbows, wrist and finger joints which interfered with his cotton picking. He saw his physician for the first time and was treated with 1,200,000 units of peincillin without results. He was then referred to the U. S. Public Health Service in St. Louis to rule out syphilis. The reported findings were repeatedly negative S.T.S. He was then referred to the Barnes Hospital with a probable diagnosis of: 1) actinomycosis; 2) pyoderma; 3) chronic osteomyelitis.

Physical examination. On admission, the physical examination revealed the temperature to be 37.8°C., pulse 84, respiration 15 and blood pressure 130/80 mm. Hg. The systemic review contributed nothing to the chief complaint except that there was a 4 to 5 cm. firm, round, hard node in the right axilla and a few smaller nodes in the right epitrochlear region. The right hand and forearm was swollen and discolored up to the elbow, showing numerous soft, fluctuant, unruptured and ruptured nodules. There were many draining sinuses from which could be obtained a thick, sticky, blood-streaked exudate or discharge (Fig. 1A).

Laboratory examination. Laboratory studies on admission showed the blood count to be as follows: hemoglobin, 10.0 gm.%; red cells, 3,860,000; white cells, 8,400; cell volume, 39.0%; platelets, normal; segmented forms, 52%; band forms, 6%; lymphocytes, 40%; monocytes, 2%. The monocytes were slightly hypochromic. The blood Kahn reaction for syphilis was negative. The urinalysis showed the specific gravity to be 1.020 and the albumin and sugar to be negative. The *Brucella* agglutination and histoplasmin tests were negative.

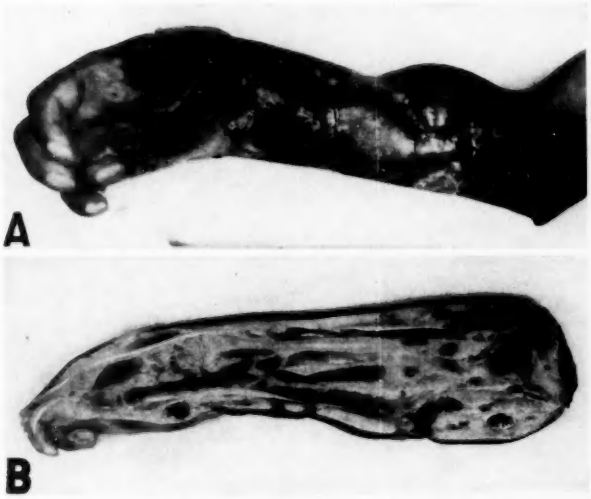

FIG. 1. *A*. Mycetoma of the hand and arm showing numerous sinus tracts and soft tissue scarring. *B*. Sagittal section of amputated arm. Note black granules particularly in mid-section.

FIG. 2. *A*. Roentgenogram of arm showing soft tissue deformity. *B*. Roentgenogram of the hand showing bone involvement.

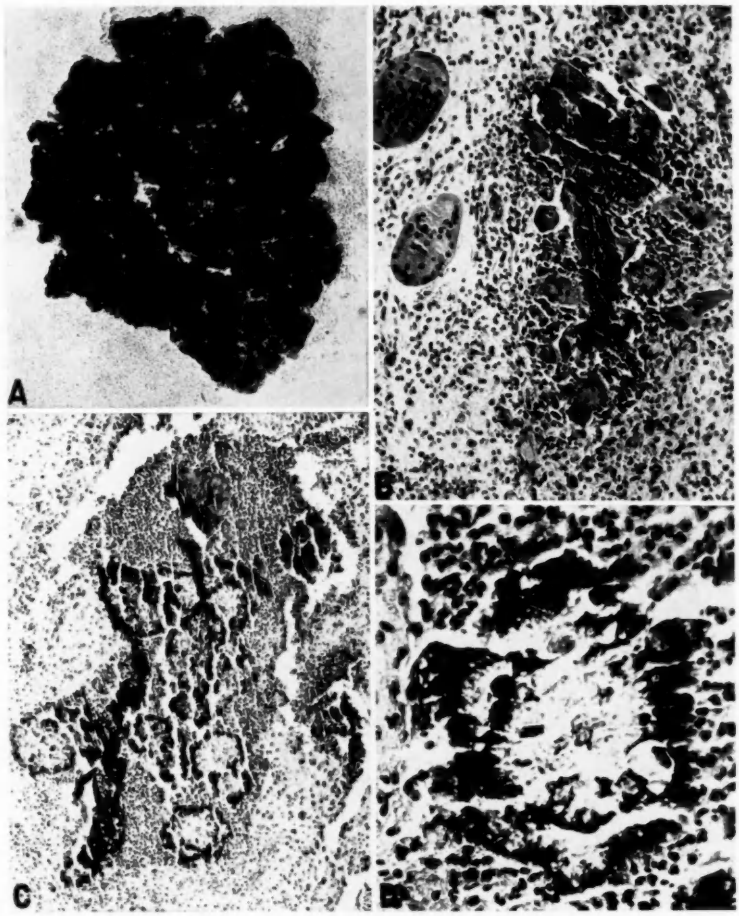

FIG. 3. A. Granule from pus mounted in potassium hydroxide (30%); × 115. *B*. Section of tissue showing foreign body type of reaction around fungus filaments. Hematoxylin and eosin; × 173. *C*. Section of tissue showing suppurative reaction around granules. Hematoxylin and eosin; × 110. *D*. Mature granule in tissue showing radiate substance. Hematoxylin and eosin; × 528.

Second strength P.P.D. gave no reaction after 48 and 72 hours. Nonprotein nitrogen was 14; fasting blood sugar, 73; total protein, 8.4; and the albumin–globulin ratio was 4.0/4.4.

A roentgenogram of the hand and arm showed deformity of the soft tissue (Fig. 2*A*). There were noticeable changes in the carpal bones with almost complete resorption of the greater multangular and considerable loss of substance in the trapezium (Fig. 2*B*). Soft tissue calcification was thought to be seen

lateral to the tibia. The x-ray findings were considered to be osteomyelitis of the carpal bones.

Smears made from the draining pus showed many polymorphonuclear leucocytes and many dispersed extracellular gram-positive cocci. There were no demonstrable acid-fast bacilli or fungus cells. On subsequent examination of smears, dark brown to black granules were noted (Fig. 3*A*). The exuding pus was shiny and sticky. Cultures were made on Sabouraud's glucose agar and on the following day isolated granules were seen on the agar slants. The granules were dark brown to black and irregular in shape, measuring up to 1 mm. in diameter. When examined in potassium hydroxide (30%) preparations, the granules appeared orange-brown in color. Cultures started to develop on the third day as a grayish growth of filaments which then became light yellow and then yellowish-brown. The organism was identified as a species of *Madurella*.

Hospital course. The patient's hospital course was uneventful. He was treated with penicillin, 3,500,000 units; terramycin, 250 mg. every six hours; saturated solution of potassium iodide, 20 drops twice daily; and solution of sodium iodide, 3 gm. intramuscularly, once daily. In spite of the administration over sufficiently long periods of these antibiotics and chemotherapeutic agents, the arm showed no response. Accordingly, the arm was amputated on November 18, 1951.

PATHOLOGY

Gross examination. The arm was removed by supracondylar amputation. Axillary and epitrochlear nodes were also removed for microscopic examination. Grossly, the specimen (arm) showed numerous sinus tracts which were chiefly on the volar aspect of the wrist. The tracts were also present in the palm. The distal part of the forearm was swollen and soft. The middle part had, on the outer edge, a depressed fibrous scar that had a sinuous form with several ramifications. Several of the sinus tracts were elevated above the surface of the epithelium and showed a whitish color. On depression, the tracts emitted a dark, granular material. On sagittal section there was found involvement of the bones of the wrist, especially of the hamate bone (Fig. 1*B*). The fifth metacarpal bone and part of the lesser triangular bone were also involved. The black or dark brown granules were arranged in regular or irregular globular masses, either discrete, with some extending up to and through the epithelial tissue, or in an almost confluent sinus tract as seen through the middle area of the arm extending into the hand.

Microscopic examination. Sections of tissue showed advanced fibrosis and chronic inflammation around large round or oval masses of the organism that appear as dark brown granules. The inflammatory process was distinguished by the large number of new capillaries in a field infiltrated by numerous plasma cells, a few scattered polymorphonuclear leucocytes, eosinophils and small round cells. Fibroblasts were also present in varied number, particularly in the areas surrounding the granules. In addition, there were apparent many large foam cells and mononuclears. The foam cells were either grouped or isolated. Some showed

large, fairly regular nuclei while in others there were large clear structures giving the foamy appearance. In many of these cells, however, there were numerous brownish globules which probably could have been fatty material containing pigment liberated by the organism in tissue.

The reaction involving the fungus directly was seen as of two types, depending on the age of the organism. In the earliest form, the fungus, which at this stage consisted of short filaments only, was engulfed by macrophages or multinucleated giant cells. As the filaments increased in size, length and quantity, the number of giant cells also increased, producing a distinct granulomatous process (Fig. 3*B*). In this older granule which now consisted of elongate filaments, there were also formed round, thick-walled cells. There was now an accumulation of polymorphonuclear leucocytes in association with the granules. Surrounding the reaction there was evident an increased number of fibroblasts and the early formation of a wall of connective tissue.

The second chief type of reaction was concerned with the mature fungus granule. The giant cells were no longer part of the reaction and were consequently replaced by a large number of polymorphonuclear leucocytes, forming a pyogenic mass which engulfed the mature fungus granules (Fig. 3*C*). This was all encapsulated by a zone of fibrosis or perhaps fibrous connective tissue. There was apparent liquefaction, as seen microscopically, which resulted in the sinus formation noticeable in the gross. The granule in the mature form was made up of spherical masses of large round, thick-walled cells, the chlamydospores.

MYCOLOGY

In tissue the organism is seen in the form of conglomerate masses, the granules. In hematoxylin and eosin stained sections these granules appear light to dark brown in color. The early state of the fungus, in tissue, consists of bundles or fascicles of hyphae or filaments which measure approximately 3 to 4 microns in diameter. The filaments in turn become converted into round or ovoid groups of spherical, thick-walled, dark brown cells, the chlamydospores, which measure approximately 6 to 12 μ in diameter (Fig. 3*D*). Surrounding the granules, composed of these mature cells, is the substance known as radiate material which is eosinophilic. In the pyogenic, often blood-streaked exudate obtained from the sinuses, the organism was seen in masses of small, hard, dark brown granules measuring approximately 1 mm. in diameter. In potassium hydroxide (30%) preparations, the granules were orange-brown in color. In these, the fungus consisted chiefly of the large, thick-walled cells at the periphery and both filaments and round cells in the central region.

Cultures were made on Sabouraud's glucose agar slants with the exuding, slimy or sticky pus, containing granules. On the third day there developed from the granules a growth of grayish filaments which a few days later became light yellow and then yellowish-brown. A brown color developed in the substratum surrounding the growth. Subcultures were made on various mediums.

Conservation agar (*Sabouraud's agar minus carbohydrate*). Growth was slower at room temperature than at 37°C. The colony was arachnoid in appearance,

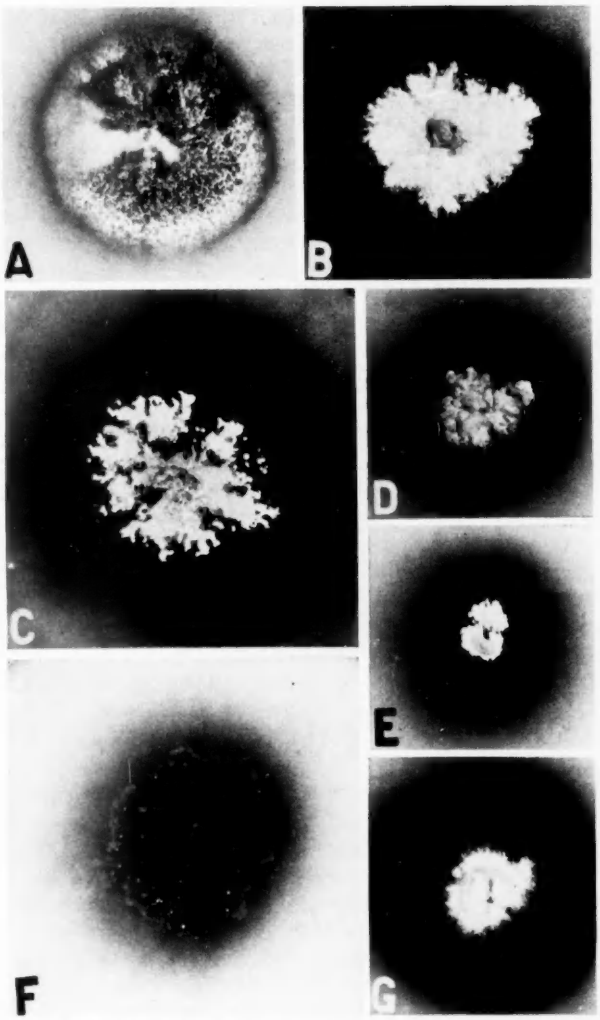

FIG. 4. Cultures of *Madurella*. *A*. Conservation agar, 23 days, 37°C., showing pin-point sized sclerotia. *B*. Sabouraud's glucose agar with neopeptone, 23 days, 37°C. *C*. Potato-dextrose agar, 23 days, 37°C. *D*. Sabouraud's glucose agar with Bacto-peptone, 23 days, 37°C. *E*. Sabouraud's glucose agar with Bacto-peptone, 23 days, room temperature. *F*. Corn-meal agar, 23 days, 37°C. *G*. Sabouraud's glucose agar with neopeptone, 23 days, room temperature.

grayish in color, with pin-pointed sized sclerotia, black in color, over the surface of the agar. Some brown pigmentation was present in the substratum but this was not as intense as on mediums containing glucose. At room temperature, after 23 days, the colony measured approximately 2 cm. in diameter. At 37°C., after 23 days, the colony measured 4.5 cm. in diameter (Fig. 4*A*).

Microscopically, the hyphae appered hyaline, septate and branching, measuring 1–4 μ; for the most part they were thin and elongate. Older hyphae showed some oidioid formation or regular and irregular oidia, some appearing dumbbell shaped. These varied from 6–9 μ, some slightly larger. Sclerotia were spread throughout the midzone of the culture (Fig. 6*C*). The young sclerotia showed an accumulation of thick-walled hyphae and oidia which were dark brown in color. The older sclerotia consisted of a more concentrated accumulation of these cells. The cells in this medium did not attain the size of similar structures found in mediums containing carbohydrate, where, however, sclerotia were not commonly found. Oidia measured approximately 6–9 μ in diameter, more uniformly 6 μ. Large chlamydospores similar to those seen on Sabouraud's glucose agar were not noted.

Sabouraud's glucose agar (with Bacto-peptone) pH 5.6. At room temperature the colony was small, approximately 1 cm. in diameter, after 23 days (Fig. 4*E*). The colony was heaped up, acuminate, cerebriform and yellowish-brown in color. Brown pigmentation was present in the substratum. At 37°C., after 23 days, the colony was approximately 1.5 cm. in diameter (Fig. 4*D*). The color of the growth was more intense than at room temperature being dark chamois. No sclerotia were seen grossly.

Microscopically, the hyphae were septate and branching, either uniform or irregular, at first smooth and hyaline, becoming yellowish-brown and granular, measuring 2–5 μ in diameter. Many filaments developed chains of oidia which were spherical to elongate in form and measured 6–9 μ in diameter (Fig. 6*A*). Young hyphae showed bifurcating, terminal portions similar to those seen with the organism causing favus. The hyphal cells became sclerotic in appearance, some having developed into intercalary chlamydospores which measured 5–15 μ, some larger. Lateral chlamydospores were also formed, some simulated large conidia and these measured approximately 6–15 μ. Terminal chlamydospores, at first spherical and smooth, became large with a rough wall and measured up to 24 microns in diameter, some larger (Fig. 6*B*). Sclerotia were not observed miscroscopically.

Sabouraud's glucose agar (with neopeptone). The colony was larger than with Bacto-peptone, measuring 2 cm. after 23 days at room temperature (Fig. 4*G*), and approximately 3 cm. after 23 days at 37°C. (Fig. 4*B*). The colony was light to dark chamois at room temperature and dark yellowish-brown to dark chamois at 37°C. The colony was cerebriform in the center with a lighter colored, arborescent, irregular growth at the periphery. No sclerotia were noted grossly.

The growth, microscopically, was somewhat similar to that on the Bacto-peptone medium. Some of the hyphae appeared thinner in diameter, 1.5 μ in diameter but older filaments measured up to 5 microns in diameter, larger in

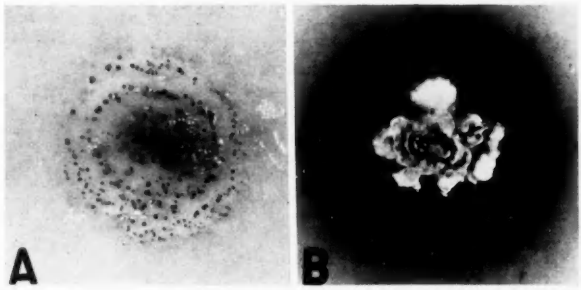

FIG. 5. *A*. Growth on corn-meal agar, 46 days, room temperature, showing concentric formation of sclerotia. *B*. Potato-dextrose agar, 31 days, room temperature, showing black sclerotia in central zone.

some instances. The young filaments were hyaline and smooth, the older hyphae were granular, larger in diameter and showed a moniliform or beaded formation of regular to irregular cells, 6–9 μ in diameter. Chlamydospores and moniliform structures were not so well defined as on the Bacto-peptone medium. Sclerotia were not observed microscopically.

Potato-dextrose agar. pH 6.2. At room temperature the colony attained a size of 1.7 cm. in diameter after 23 days. The growth was gray to grayish-brown in color, arborescent in character with some brown pigment diffusing into the medium. The central zone of the colony contained numerous black, irregular sclerotia measuring up to 1 mm. in diameter. After 31 days the colony became darker in color, more irregular and the sclerotia more prominent (Fig. 5*B*). At 37°C. the colony measured approximately 3.5 cm. in diameter after 23 days (Fig. 4*C*). The arborescent character of the colony was more accentuated and the color was deep, dirty, yellowish-brown with a more intense brown pigmentation of the substratum. Sclerotia were not observed grossly in cultures grown at 37°C.

Microscopically, the filaments appeared irregular and curved, measuring approximately 2–5 μ in diameter. The hyphae developed chains of smooth to granular, uniform to irregular, spherical to sclerotic cells, approximately 6 μ in diameter. Many cells were dumb-bell shaped. Many cells appeared to project laterally, some on short pedicels analogous to conidia. Other spherical cells appeared terminally, measuring approximately 6–22 μ in diameter. These cells, predominantly chlamydospores, did not, in general, attain the size of those seen on Sabouraud's glucose agar with Bacto-peptone. An occasional large, granular cell was found which measured 27 μ. There were also seen clumps of thick-walled cells, apparently the nucleus for the formation of the sclerotia. The sclerotia were made up of large, thick-walled dark brown cells and dark brown, short, thick filaments.

Corn-meal agar, pH 6.0. Growth appeared in the medium, chiefly submerged. At 37°C. the colony measured 3.2 cm. in diameter after 23 days growth (Fig. 4*F*). The growth was brown in color due perhaps for the most part to the diffused

brown pigment in the substratum. There were a few irregular sclerotia, dark brown in color, spread throughout the growth. At room temperature, after 23 days, the colony measured 2 cm. in diameter. It was completely submerged in the medium, clear in appearance and the medium, as in the case of the colony grown

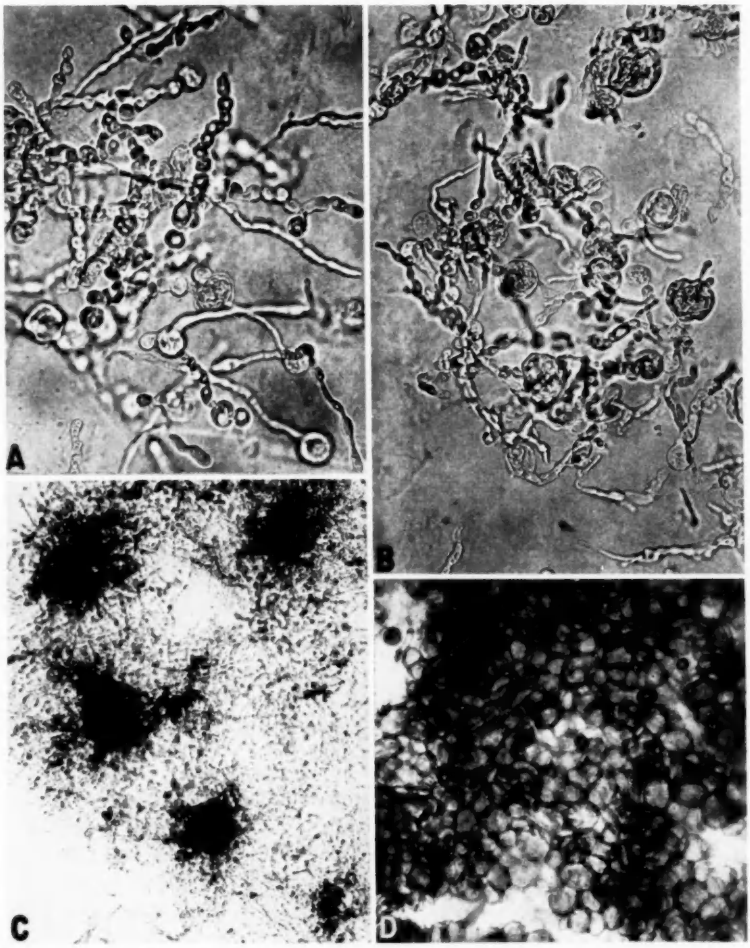

FIG. 6. *A*. Slide mount from Sabouraud's glucose agar, three weeks after inoculation; × 630. Note chain of oidia and terminal chlamydospores. *B*. Slide mount from same medium, second subculture; × 525. There are fine and coarse, irregular filaments and numerous chlamydospores. *C*. Early sclerotia formed on conservation agar, 18 days after inoculation; × 185. *D*. Wall of sclerotium mounted in potassium hydroxide (30%), grown on corn-meal agar; × 660.

at 37°C., appeared to be lysed. The mycelium was hyaline as compared with the brown color of the colony grown at 37°C. Submerged in the medium, however, were numerous dark brown to black, irregular sclerotia, pin-point to somewhat larger but less than 1 mm. in diameter. There was only a slight amount of pigment diffused into the medium. After 46 days at room temperature, the sclerotia became more apparent on the surface of the medium as concentric rings (Fig. 5*A*).

Microscopically, the hyphae were fairly uniform in appearance, showing very few oidioid structures and only a few terminal chlamydospores. The hyphae were of small diameter but increased in size as they approached the sclerotia where they changed from the narrow, hyaline type to a somewhat granular thick-walled, dark brown filament. Accordingly, they varied in diameter from 1–5 μ. The oidia were extremely few in number and when found in older filaments they measured approximately 6 μ in diameter. The terminal chlamydospores varied in size but seldom exceeded 12–15 μ in diameter. The early sclerotium consisted of a mass of dark brown oidioid cells and thick-walled, dark brown hyphae. The mature sclerotia were formed within the medium. They consisted of an outer sheath or wall made up of fairly uniform dark brown, thick-walled cells measuring approximately 8 μ in diameter (Fig. 6*D*). These were formed by the clumping together of oidia. The inner portion of the sclerotium consisted of filaments and oidia which undoubtedly had become chlamydospores.

Czapek's agar. pH 4.4. The growth was completely submerged in the substratum, barely visible except by transmitted light and showed a light brown color with an arborescent type of growth. At 37°C. the colony measured 3.2 cm. in diameter after 23 days. Scattered throughout the central area were numerous pin-point sized, irregular, dark brown sclerotia. At room temperature, after 23 days, the colony measured 1.5 cm. in diameter.

Microscopically, the submerged hyphae were sparse and uniformly long, narrow, hyaline filaments measuring 1–3 μ in diameter. Approximately 0.5 cm. from the center of the inoculum, within the agar medium, forming an irregular peripheral ring, were seen minute, pin-point sized black sclerotia. These consisted of clumps of dark brown, thick-walled oidia and filaments similar in dimension to those seen on corn-meal agar. There were very few filaments connecting the sclerotia.

Wort agar. pH 4.8. Growth was extremely poor both at room temperature and at 37°C., with only a slight increase over the size of the inoculum after 46 days. The colony was dark brown in color and moruloid in character.

Microscopically, the hyphae were chiefly short and thick-walled, measuring for the most part, 4–6 μ in diameter with some irregular filaments as much as 9 μ. The hyphae were granular, showing early formation of numerous oidioid cells and many chlamydospores measuring 6–24 μ in diameter, some larger. The microscopic appearance was apparently compatible with unfavorable growth conditions.

Sabouraud's glucose broth. pH 5.5. Large floccules developed at the bottom of the flask. Growth at 37°C. was somewhat better than at room temperature.

A single floating colony was observed on the broth surface. The broth became brown due to the diffusible pigment. The microscopic picture was analogous to that seen on the agar. There were, however, more chlamydospores.

Starch agar. pH 6.8. Deep brown pigmented colonies developed. There was definite hydrolysis of the starch.

Gelatin. The gelatin was not liquefied even after many weeks of growth. The fungus grew on the surface forming a dark brown colony with several dark brown to black sclerotia. Dark brown pigment diffused into the gelatin.

Litmus milk. The milk was not acidified or curdled and became dark brown in color.

Serum (inspissated). There was no liquefaction and growth was poor.

CLASSIFICATION OF ORGANISM

The classification of the species of *Madurella* is still in a state of confusion. As a result of the work of several investigators, however, some order is being brought to the chaotic nomenclature.

The genus *Madurella* was created by Brumpt (1905) using as the type species the organism described as *Streptothrix mycetomi* by Laveran (1902). In both instances the species and genus differentiation was based on the observation of granules and not actual cultures. Accordingly, the species, at least, was considered to be a *nomen dubium* by Vuillemin (1918–1919) and by MacKinnon and his associates (1949a, 1949b).

At approximately the same time, Chatterjee (1911) and Brault (1911) isolated and described *Madurella mycetomi* in culture. Brault (1912) enlarged upon his description and Noc and Jouenne (1922) published a detailed cultural description of the fungus. Puyhaubert and Jolly (1918) published a case with perineal involvement and gave some cultural descriptions of the fungus (1919, 1920). The characteristics attributed to *Madurella mycetomi* apparently are sufficient to establish the specificity of the organism. However, not all published reports are in complete agreement as to the details of these characteristics.

According to Brault, *M. mycetomi* was described as follows: "Mycelium grayish-white, yellowing with age, darkening sugar culture mediums. Oidia of variable dimensions, 2–5 microns. Sclerotia black, sterile, 0.5–1 mm. in diameter, found in the interior of the culture medium. Able to invade in man, skin, bone, muscle and the adjacent tissue; producing a mycetoma with black granules; the granules are small, hard, round, more or less verrucous, with a morphology simulating that of the sclerotia found in the culture. Animal inoculations are negative."

This description appeared inadequate and was, therefore, enlarged by Yazbek (1920) and by Noc and Jouenne. Gammel (1927) and recently MacKinnon (1951a) have summarized the data on this organism. By combining the characteristics described by these various investigators, *M. mycetomi* would be described as follows: *Madurella mycetomi* (Laveran) Brumpt, 1905. In tissue there are produced hard, dark brown to black, discrete or conglomerate granules, round, irregular or verrucous in form, measuring approximately 0.5–2 mm. in diameter. Granules comprised of brown, septate hyphae measuring 2–5 μ in di-

ameter with hyphal cells measuring approximately 3–15 μ in length. Chlamydospores both intercalary and terminal are also present, measuring 7–12 μ in diameter but may become up to approximately 25 μ. An interstitial brown substance may be present in spaces among hyphae.

On Sabouraud's glucose agar the organism produces a grayish-white mycelium which becomes yellow and then brown with age and covered with a yellowish-brown aerial mycelium. The colony attains a size of 1–2 cm. in diameter. The center of the colony becomes verrucous and raised, and develops radiating furrows with age. On various carbohydrate mediums a brown pigment diffuses into the substratum. The vegetative hyphae are septate, irregular or regular with hyphal cells approximately 1–5 by 7–26 μ. Chlamydospores are either terminal or intercalary, measuring approximately 7–20 μ in diameter with some measuring up to 26 μ or even larger. Sclerotia measuring 0.5–1 mm. are present in Sabouraud's proof medium and in potato water and peptone broth, sometimes numerous within the medium. The culture may lose this property.

The optimum temperature is approximately 37°C. Gelatin may or may not be liquefied. Milk is usually not coagulated.

The organism produces in man mycetoma with involvement of the skin, muscles, bones and joints.

Unfortunately, it is difficult to ascertain except by the published descriptions whether in each case the authors were dealing with the same species. In many instances the original cultures were lost. The discrepancies in the description reported by Brault, Yazbek and Noc and Jouenne might mean different interpretations of the same species or perhaps even different species. MacKinnon for example lists the chlamydospores as measuring 7–20 μ whereas Brault and also Negroni, Fernandez and Daglio (1947) consider them to be 7–12 μ and Noc and Jouenne, 16–25 μ. Noc and Jouenne further describe moniliform filaments whereas Yazbek described fine, regular filaments.

If the combined characteristics of Brault, Yazbek and Noc and Jouenne were to be considered as the description of *M. mycetomi* then we would have to include as synonyms of the organism *M. tozeuri* (Nicolle and Pinoy, 1908), *M. americana* Gammel (Pinoy, 1912), *M. ikedai* Gammel (Miskdjian and Thatcher, 1926) and perhaps several others as has been done by MacKinnon.

In the world literature there have been described several species of *Madurella*. Some of these may possibly be synonymous while others undoubtedly are distinct species. On the basis of published descriptions and illustrations it is difficult to separate out species as *M. ramiroi*, *M. oswaldoi*, *M. tozeuri*, *M. americana* and *M. ikedai* from *M. mycetomi*.

Negroni, Fernandez and Daglio separate the principal species on the basis of gross cultural characteristics. Under the cottony type they list *M. mycetomi*, *M. oswaldoi* and *M. tabarkae*. The powdery forms consist of *M. tozeuri* and *M. americana*. The faviform species are made up of *M. ramiroi* and *M. lackawanna*. These, however, are not constant characteristics since on different mediums and at different temperatures the gross appearance may vary. This holds true for *M. mycetomi* and *M. americana* which may become faviform or cerebriform.

In the United States there have been described three species of *Madurella*.

Of these, *M. lackawanna* is sufficiently different from all other species of the genus and from the organism described here and thus need not be considered here. The remaining two, *M. americana* and *M. ikedai*, however, need further consideration. According to Gammel, both species are fairly slow growers, both produce grayish-white mycelium which becomes yellowish-brown and both produce sclerotia on the surface of Sabouraud's preservative agar, with *ikedai* forming many deep in the medium. The filaments of both species vary from 1–5 μ in diameter. *M. ikedai* forms numerous moniliform oidia whereas *M. americana* shows rare formation of oidia. Chlamydospores appear late in *M. americana* but are abundant in *M. ikedai*. In many respects the similarity to *M. mycetomi* as described by Noc and Jouenne is rather apparent. The microscopic morphology of both *M. americana* and *M. ikedai* resembles closely the morphology of *M. mycetomi* as illustrated by Brumpt. In all probability both *M. americana* and *M. ikedai* should be classified as one species under the name *M. mycetomi*.

According to Negroni, Fernandez and Daglio *M. americana* is described as producing, on Sabouraud's glucose agar powdery, yellow colonies which become darker with age and measuring approximately 1.5 cm. in diameter. Sclerotia are present in culture. In the granules the mycelial elements measure 10–14 by 2.5–6.5 μ and chlamydospores 21–27 μ in diameter. In culture the elements measure 10.1 by 2.3 μ. The fungus grows best at 37°C. and liquefies gelatin slowly. This description, with slight variation, agrees with that of Noc and Jouenne. Furthermore, this description, is in general agreement with that of the organism isolated from the arm reported here.

It must, therefore, be concluded that the fungus described in this paper has much in common with *M. ikedai*, *M. americana* and *M. mycetomi*. The possibility that there are different strains showing variations of the one species should be given prime consideration.

According to Dodge (1935) the genus *Madurella* is placed in the tribe Toruleae. The Toruleae are characterized as follows: "Mycelium usually dark colored, often black, spores usually dark colored; no differentiation of sporophores; multiplication by arthrospores and chlamydospores, rarely sprouting." The genus *Madurella* is a difficult one to classify properly since the morphology of the various species attributed to it has not been adequately studied. Dodge lists eight species, some of which, as has been discussed, might well be synonymous with others.

The genus *Indiella* appears to be closely related to *Madurella*. Here the organisms produce colorless or white granules, forming a white thallus and is consequently included in the Mucedineae. Dodge lists three species parasitic on man.

Ciferri and Redaelli (1941) combine the species of *Madurella* and *Indiella* in the one genus *Madurella*. They further create subgenera based on color and whether the granules are present in tissue alone or in tissue and in culture mediums. Their classification is the following:

1. *Madurella* subg. *Eumadurella* Ciferri and Redaelli, 1941. Sclerotia of varying color produced in laboratory mediums and in living tissue.

   Type: *Madurella* (*Eumadurella*) *mycetomi* (Laveran) Brumpt, 1905.

2. *Madurella* subg. *Pseudomadurella* Ciferri and Redaelli, 1941. Sclerotia black or dark produced solely in living tissue.
   Type: *Madurella* (*Pseudomadurella*) *oswaldoi* Parreiras Horta, 1919.
3. *Madurella* subg. *Rubromadurella* (Talice) Ciferri and Redaelli, 1941. Sclerotia red or purple produced solely in living tissue.
   Type: *Madurella* (*Rubromadurella*) *langeroni* Talice, 1935.
4. *Madurella* subg. *Indiella* (Brumpt) Ciferri and Redaelli, 1941. Sclerotia white or bright colored produced solely in living tissue.
   Type: *Madurella* (*Indiella*) *mansoni* Brumpt, 1906.

According to this classification, the organism described in this paper would be placed in *Madurella* subgenus *Eumadurella* either as or with *M. mycetomi.*

The acceptance of several of the species as authentic is a matter for further study. Likewise, the acceptance of the several subgenera of Ciferri and Redaelli is subject to dispute. Much of the discrepancy in the species descriptions may be attributed to a lack of correlation with other organisms. MacKinnon has attempted to do this but only with a limited number of species and the specificity of some of these may be disputed. *Madurella* (*Rubromadurella*) *langeroni* for example never was cultured, the description being based only on the granule seen in tissue. This alone would put some doubt on the validity, even if it was acceptable, of the species, perhaps of the genus and certainly of the subgenus. In short, this would be a *nomen dubium.* Actually, the genus needs a complete, comprehensive and detailed analysis.

As to the organism reported in this paper, its similarity to *M. ikedai*, in many respects, is quite evident. In a personal communication, Gammel now feels that *M. ikedai* is similar to *M. americana* and that both perhaps should now be known as *M. americana.* A strain of *Madurella* labelled *M. americana*, now in the collections of several mycologists, however, does not appear to be similar to *M. ikedai.* Gammel further feels that that is not the strain isolated and distributed by him. According to the published description, there is also a possibility that it may be *M. mycetomi.* Strains identified as *M. mycetomi* in the British mycological collections certainly are not identical with or similar to the strain reported here. An attempt is being made to gather together all the available species of *Madurella* to help clarify the taxonomic difficulties in the genus. Until this work is completed, it would seem best to temporarily consider the organism described here as or closely related to *M. ikedai* and possibly synonymous with *M. americana* and/or *M. mycetomi.*

COMMENT

The history of mycetoma has been well presented by Castellani and Chalmers. The clinical (Boyd and Crutchfield, 1921; Thompson, 1927; Moore, 1939), pathological (Symmers and Sporer, 1944; Moore, 1945) and much of the mycological (Gammel, 1927; MacKinnon et al., 1949; MacKinnon, 1951a, 1951b; Brumpt, 1949) aspects of mycetoma have been rather well described in these and numerous other publications. A repetition of the general data on this disease, therefore, would be not only time- but also space-consuming.

TABLE 1

Mycetomas with hand and/or arm involvement

AUTHOR AND YEAR	COUNTRY	AGE	COLOR	SEX	DURATION OF LESION	LOCATION OF LESION	BONE INVOLVEMENT	COLOR OF GRANULE	ORGANISM	REMARKS
Kanthack, 1892	England	?	?	?	?	Hand and arm	?	Yellow and black	*Nocardia* (?)	Not cultured
Bocarro, 1893	?	?	?	?	?	Hand	?	?	?	Three cases out of 100
Ocaranza, 1914	Mexico	Adult	W	M	1½ yr.	Hand	Yes	Black	*Nocardia* sp.	
Nicolle and Blanc, 1920	Tunisia	19	Native	F	15½ yr.	Left hand, right leg and foot	Yes	Black	*Madurella* sp.	
Guy, 1920	U. S. A.	50	W	M	6 mo.	Left hand and arm	?	?	*Nocardia* sp.	
Montpellier, Gouillon and Lacroix, 1921	Algeria	30	Native	M	Since childhood	Right arm	No	White	?	Not cultured
Puestow, 1929	U. S. A.	19	W	F	Recurrent 6 yr.	Nose, cheek, right ear, right forearm	No	Black	*Aspergillus nidulans*	
Mazzini, 1934	Argentina	26	W	M	15 yr.	Left palm	Yes	Black	*Actinomyces* (*Nocardia*) *melanico*	
Talice, 1935	Uruguay	Adult	W	M	?	Finger	No	Red	*Rubromadurella langeroni*	Not cultured
Redaelli and Ciferri, 1942	Italy	Adult	W	M	?	Right forearm, arm and hand	?	?	*Actinomycete*	Case of Agata
Milian, 1943	Poland	30	W	F	2½ yr.	Left hand and lower forearm	Yes	?	*Actinomycete*	Not cultured
Symmers and Sporer, 1944	U. S. A.	67	W	M	7 yr.	Right hand	Yes	Black	*Phialophora jeanselmei*	
Lacaz, 1945a	Brasil	Adult	Negro	M	?	Back of right hand	Yes	?	Actinomycete	Case of de Magalhães and Aroeira Neves
Lacaz, 1945a	Brasil	57	W	M ♂	1 yr.	Finger of left hand	Yes	Yellowish-white	*Nocardia brasiliensis*	
Lacaz, 1945b	Brasil	60	W	M	?	Right arm and forearm	Yes	?	*Actinomyces albus* (?)	
Myerding and Evert, 1947	Mexico	39	W	M	17 yr.	Left hand	?	Black	*Madurella* sp.	
Sutton and Sutton, Jr., 1952	U. S. A.	Adult	W	?	5 mo.	Forearm	?	?	*Nocardia* sp.	Case of Sweitzer
Moore, 1953	U. S. A.	64	Negro	M	15 yr.	Right hand and arm	Yes	Black	*Madurella ikedai* (?) (*M. mycetomi, M. americana*)	

Although the number of organisms causing mycetoma is rather large, there are in addition other disease processes caused by fungi, bacteria and spirochetes which may at times simulate mycetoma. Notable examples of course are chromomycosis (chromoblastomycosis), sporotrichosis, tuberculosis and syphilis. The finding of the causative agent in each case, therefore, becomes of extreme diagnostic and prognostic value.

It is well known that the actinomycetes, particularly the aerobic nocardias, contribute many instances of mycetoma in the literature. Since the organisms of this group do not tend to remain localized to the original site, as in the case of primary cutaneous lesions, the hand and/or arm have been involved in a relatively greater number of patients than have similar lesions caused by organisms belonging to other genera. In some instances such cases, in addition to several caused by other genera, have been reported as maduromycosis. This is incorrect since only those lesions caused by species of *Madurella* should be so classified.

An attempt was made to list as many cases of mycetoma involving the hand and/or arm, regardless of genus or species, as could be obtained from the available literature. These are listed in Table 1. Very little information could be found on some of these cases.

One of the earliest reports of mycetoma of the arm is that of Kanthack (1892) who presented two specimens, an amputated hand and a foot, at a meeting of the Pathological Society of London. Both were considered to be due to an actinomycete, one having yellow granules and the other the black variety. The organisms were not cultured.

In a review of 100 cases, Bocarro (1893) found only three cases of mycetoma involving the hand. The causative organisms were not reported.

Ocaranza (1914) reported four cases of mycetoma, three of the foot and one of the hand. The patient was an adult Mexican ranch worker who had developed the infection as a result of traumatic inoculation with a mesquite spine. This case had been previously reported by Cicero (1912). The organism was supposedly a *Nocardia*.

Probably the first *Madurella* infection of the hand was the case reported by Nicolle and Blanc (1920). The patient was a 19-year-old Tunisian young woman who had developed the infectious process at the age of three years. The lesions involved the right foot and leg and the left hand. There was bone involvement of the hand. The granules were black and the organism was identified as a species of *Madurella*.

Guy (1920) reported a sporotrichotic-like lesion of the left hand and arm caused by a *Nocardia*. The patient was a 50-year-old coal miner who had developed the infection in the left thumb six months previously. The clinical features, although resembling sporotrichosis, could just as well have been considered as a mycetoma. The organism was cultured and identified as a *Nocardia*.

Boyd and Crutchfield (1921) made a fine review of mycetoma in North America and listed 32 cases occurring up to 1921 with only one affecting the hand, that reported by Ocaranza. In the same year, Montpellier, Gouillon and Lacroix (1921) reported a mycetoma of the right arm resulting from an inoculation with

a spine, in a 30-year-old Senagalese man. The lesion was present from childhood and involved the posterior surface of the right arm a little below the epitrochlear and consisted of a firm, ovoid nodule the size of an almond, which was painful. White granules were obtained but unfortunately the organism was not cultivated.

Puestow (1929) reported a case of maduromycosis in a 19-year-old white woman, a university student and native born New Yorker, with involvement of the nose, cheek, area behind the right ear and extensor surface of the right forearm. The lesions had been recurrent for a period of approximately six years. The granules were black in color, appearing like caviar. The organism was cultivated and identified as *Aspergillus nidulans*.

In view of the fact that the organism was *A. nidulans*, this should not be classified as maduromycosis but rather as aspergillosis or mycetoma due to *Aspergillus*.

Mazzini (1934) presented before the Surgical Society in Buenos Aires a case of mycetoma involving the palm of the left hand. The patient, a 26-year-old man, had developed the lesion 16 years previously without any incidence of trauma. The resulting tumefaction became enlarged to produce a typical mycetoma with involvement of the fourth and fifth fingers. Black granules were obtained from the exudate and the organism was considered to be an actinomycete and was called *Actinomyces melanico*.

In the following year, Talice (1935) reported the case of an adult white man, an Uruguayan, with a nodular involvement of one of the fingers. The nodule was excised by the surgeon and sent to the laboratory for diagnosis. The tissue showed red granules characteristic of *Madurella*. Although cultures were not made, since the available material had been fixed, the organism, from its parasitic appearance, was named *Rubromadurella langeroni*.

Redaelli and Ciferri (1942) published Volume V of Trattato de Micopatologia Umana. On page 142, Figure 15 illustrates the case of a patient of Agata. This is a case of actinomycosis characteristic of mycetoma with involvement of the right forearm presenting well developed tumefactions and deformities with extension of the process to the arm and to the dorsum of the hand.

In the following year, Milian (1943) reported a mycetomatous lesion of the left hand and lower forearm in a 30-year-old Polish woman who was seen in France in 1932. The lesion which was 2½ years in duration was apparently caused by an actinomycete seen only in tissue sections. Granules were not observed in pus and cultures were not obtained.

Symmers and Sporer (1944) reported a case of mycetoma of the right hand. The patient was a 67-year-old white man, a native of New York, who had had the lesion for seven years when first seen. There were noted multiple nodules and superficially healed sinuses capped by black material on both sides of the hand. Roentgenograms revealed bone involvement. Black granules were obtained from the draining sinuses. On the basis of the identification of the causative fungus, there has been some dispute as to what the disease should be called. Clinically, the lesion was that of mycetoma. Mycologically, the organism has been described by Emmons (1945) as *Phialophora jeanselmei* making it synonymous

with *Torula jeanselmei* described by Langeron (1928) and isolated by Jeanselme, Huet and Lotte (1928) from a mycetoma of the foot. On this basis, the lesion would be a mycetoma. According to Lewis, Hopper, Sachs, Cormia and Potelunas (1948) the fungus should be placed in *Fonsecaea* along with *Hormodendrum* and *Phialophora* as suggested by Carrion (1942). This would make the disease compatable with chromomycosis (chromoblastomycosis). The present concept among mycologists is that *Fonsecaea* is synonymous with *Phialophora*.

There are several features, however, which would make this lesion a mycetoma rather than chromomycosis. In the first place, the lesion clinically was that of mycetoma. The infectious process was deep in the cutis and there was bone involvement as seen roentgenologically. Bone involvement does not usually occur with chromomycosis. In the second place, the organism in tissue was in the form of granules. Granules do not occur in chromomycosis but one sees dark brown cells either singly or in clumps but not in the form of granules. In the third place, the foam cells described by Symmers and Sporer in the tissue sections, although present in several pathologic states is characteristic of some mycetomas and not of chromomycosis. The foreign body type of reaction is not limited to any one mycotic process. Although frequently seen in chromomycosis, the process is also seen in mycetoma caused by various organisms.

On the other side of the ledger is the similarity of *Phialophora jeanselmei* to organisms associated with chromomycosis. *P. jeanselmei*, however, appears to be an unusual fungus. Berger (1944) and Berger, Beaudry and Gaumond (1945) reported a yeastlike fungus as causing the first Canadian case of chromomycosis. The organism was described as producing pasty, smooth, dark brown to black colonies and was named *Candida nigra canadensis* (*Torula bergeri*). In tissue it produced dark brown yeastlike cells. The culture, however, had all the characteristics attributable to *Phialophora jeanselmei*. On two occasions, a similar organism was isolated by two separate dermatologists from Florida, from lesions typically those of chromomycosis and sent to my laboratory as the cause of the disease. These were two strains of *P. jeanselmei*. Again, on two separate occasions, *P. jeanselmei* was isolated from skin lesions by a St. Louis dermatologist but in neither case could the lesion be proved to be caused by the fungus. In other words, both latter strains were contaminants.

In their introduction, Symmers and Sporer mentioned that they found reports of four cases of maduromycosis involving the hand, in the literature from the years 1876 to 1941. Since no references were furnished, either in the paper or in correspondence with Symmers, it is difficult to know just what these cases were.

Lacaz (1945a) illustrated two cases of mycetoma, one on the back of the hand (observation No. 8, Figure 34) and the other of the finger (observation No. 10, Figures 39–41) both caused by actinomycetes (*Nocardia brasiliensis* in the finger case). In another publication of the same year, Lacaz (1945b) illustrated an actinomycotic mycetoma of the right arm and forearm which roentgenologically showed osteopereostitis and osteomyelitis occurring in a 60-year-old white man from São Paulo, Brasil (observation No. 28, Figure 4). In the same publication

he further reported an actinomycete described by da Fonseca from the Instituto Oswaldo Cruz, Rio de Janeiro, which was isolated from a mycetoma of the arm and forearm and which produced small, yellowish-white granules.

Negroni, Fernandez and Daglio (1947) reviewed the world literature on mycetoma caused by *Madurella* and listed 30 cases, with only one case, that of Nicolle and Blanc, with involvement of the hand.

In the same year, Meyerding and Evert (1947) reported four cases of mycetoma with one involving the hand and caused by *Madurella*. The patient was a 39-year-old native Mexican merchant who had a swelling of the left hand for 17 years. While working on a farm, a pimple developed in the left palm which enlarged and discharged a purulent material from time to time. There was an indurated area over the base of the left first metacarpal bone in which there were about 10 small draining sinuses from which exuded a purulent material containing black granules. The granules were proved to belong to *Madurella*.

Sutton and Sutton, Jr. (1952) in Tice's Practice of Medicine, illustrated a case of mycetomatous-like involvement caused by a *Nocardia*. The lesion of the forearm was of five months' duration.

Undoubtedly there have been other instances of mycetoma or mycetoma-like lesions of the hand and/or arm throughout the world. Some of these were reported perhaps in journals not readily accessible and others which never have been reported. In one instance a clinical diagnosis of mycetoma of the hand was made in a patient in 1913 at the Barnard Free Skin and Cancer Hospital. The lesion began one year previously as a small red papule. In spite of treatment there developed sinuses which discharged a purulent material. Granules were not observed and the pathologist reported chronic inflammation. There was no evidence of syphilis or of a neoplasm. Accordingly, no definite diagnosis was made.

Although it is felt that the cases of mycetoma or mycetoma-like lesions of the hand and/or arm listed here are not complete, they represent those that could be found in the available literature. An analysis of these reported instances of mycetoma shows that there are a total of 21 cases including the one being reported here. Of these the etiology of three was not indicated; 11 were due to or probably due to an actinomycete; one was attributed to a white-grained fungus, probably *Monosporium*; one was caused by *Aspergillus nidulans*; one by *Phialophora jeanselmei*; one possibly was caused by a red-grained *Madurella*, although the fungus was not cultured. In two instances the species of *Madurella* was not identified. The fourth instance of *Madurella* infection is the one reported in this paper.

The distribution of the cases regardless of etiology is world-wide. Mycetoma of the hand and/or arm caused by a *Madurella*, which is of particular significance in this paper, was observed in natives of Africa, Uruguay, Mexico and the United States. The patient presented in this paper actually represents the first reported instance of a hand and arm mycetoma in a native of the United States caused by a species of *Madurella*. It is the third case in the United States caused by a true fungus as contrasted with the actinomycetes including *Nocardia*, which are considered to be false fungi, and the sixth such case in the world literature.

SUMMARY

A report is made of mycetoma of the hand and arm of 15 years' duration in a 64-year-old colored man who had lived on farms in Mississippi and Missouri all his life. A clinical and pathologic study of the lesions is presented. The histopathologic study revealed two types of reaction of the tissue to the organism, the one a foreign body type and the other a suppurative type. The organism forms dark brown to black granules in tissue. Gross and microscopic studies were made of the organism on various mediusm, as well as studies of its various biochemical reactions. The fungus is classified temporarily as *Madurella ikedai* Gammel, 1927, possibly synonymous with *M. americana* and *M. mycetomi*.

A review of the available world literature revealed that there have been at least 21 cases reported (including the present report) of mycetoma or mycetoma-like lesions involving the hand and/or arm. Of these, the etiology of three was not indicated; 11 were due to, or probably due to, actinomycetes; one was caused by a white-grained fungus; one was attributed to *Aspergillus nidulans*; one to *Phialophora jeanselmei*; one possibly to a red-grained *Madurella*; and two by species of *Madurella*. The case presented here represents the fourth *Madurella* infection of the hand and/or arm in the world literature and the first in a native of the United States.

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SPUTUM EXAMINATION IN THE DIAGNOSIS OF BILHARZIASIS OF THE LUNGS

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Schistosome infection of the lungs accompanied by cardiac complications is now regarded as a well established clinical syndrome. The first case in Egypt was discovered by Azmy and Effat (1932) and subsequently other cases were discovered. Shaw and Ghareeb (1938) studied in detail the pathological findings of the disease and estimated the incidence of Ayerza's[1] disease due to schistosome infection as 2 per cent of their series of 282 patients suffering from bilharziasis and as 6.3 per cent of their pulmonary patients. Meira (1942) in Brazil has described the types of pulmonary complications in schistosomiasis mansoni.

La Rous (1929) pointed out that in sheep infected with *S. matthei* the lungs showed the constant presence of pseudotubercles around eggs. However the presence of schistosome eggs in human lungs was described as early as 1885 by Belleli in Alexandria. Turner (1908) in South Africa found that 64 per cent of human cases dying of pulmonary complaints showed *S. hematobium* in their lungs, while in cases dying from other complaints, eggs were only found in 33 per cent. Erfant (1950) described a case of hepatosplenomegaly showing schistosome eggs in the sputum.

Since 1944 we have been interested in the subject of bilharziasis of the lungs. In the last few years we have been trying to do routine chest x-ray examinations of all the cases of bilharziasis with especial emphasis on those showing pulmonary symptoms.

We started also in 1950 making routine sputum examinations to detect the presence of schistosome eggs in such cases. Some cases presented a picture of hepatosplenomegaly associated with cardio-pulmonary symptoms, but others were simple cases of urinary bilharziasis, most of which however complained of cough. The 24-hour sputum was collected, 5 per cent of caustic soda was added and allowed to remain for two hours, after which the sediment was examined. This procedure was repeated 3–6 times on several consecutive days in 62 cases of bilharziasis, revealing schistosome eggs in 22. Eggs of *S. hematobium* were discovered in 20 of these while eggs of *S. mansoni* were found in 2. Four of the cases developed hemoptysis, and in these a considerable number of schistosome eggs were demonstrated.

Clinical description of the cases. In the series of 22 cases the urine was positive

[1] The name Ayerza's disease was given originally in South America to a condition of primary pulmonary arteriosclerosis, which may be occasionally associated with schistosome infection of the lung. These cases are usually characterized by marked cyanosis. The term has also been given in Egypt to cases of pulmonary schistosomal infection characterized by cardio-pulmonary symptoms. A better term however is cardio-pulmonary schistosomiasis or bilharzial cor pulmonale, for the latter term explains the exact mechanism of heart failure in this syndrome. The name Ayerza is objectionable because cyanosis is only present as a terminal event when there is advanced heart failure.

for *S. hematobium* in 18 cases and the stools were positive in 6 cases. In 4 cases with a history of schistosome infection, the sputum was positive although the urine and stools were negative. Most of the patients complained of cough, sometimes with hemoptysis, and of dyspnea on exertion, with or without cyanosis.

Examination of the heart usually showed the apex beat in the normal position; in some cases the heart was moderately enlarged, and a picture of Ayerza's disease was present in 4 cases in which there were signs of diastolic shock in the 2nd left intercostal space together with an accentuation of the 2nd pulmonary sound. As for the lungs, it was noted that in 12 cases there were few physical signs in the chest in the form of rhonchi or crepitations. Five patients complained of asthmatic attacks. The liver and spleen were enlarged in 14 cases and the former was usually cirrhotic. Ascites was present in 4 cases. Most of the cases (18 out of 22) showed a picture of severe anemia in which the hemoglobin was between 20–40 per cent.

The radiological findings in most of the cases presented the features described as characteristic of lung bilharziasis. There was intensification of the shadows of the small arteries, especially the basal, which become wider in diameter with dense shadows, tortuous course, irregular hazy outline and beaded appearance due to the presence of bilharzial nodules. Some cases presented enlarged hilar shadows which were usually dense. Some early cases showed mitralization of the heart, while late cases showed the typical picture of "cor pulmonale." Routine screens in the oblique view were done to exclude congenital heart disease.

DISCUSSION

It is interesting to find such a high percentage of cases showing schistosome eggs in the sputum. The more frequent presence of *S. haematobium* eggs in the sputum in our cases is worthy of comment. In urinary tract infection the eggs of *S. haematobium* are readily carried to the right side of the heart and lungs because of the direct communication between the vesical plexus and the systemic circulation. This is supported by our present findings. The fact that most of the positive cases as previously mentioned did not show the picture of Ayerza's disease is doubtless due to the fact that *S. haematobium* infection of the lung is less likely to cause diffuse vascular lesions than *S. mansoni*. In *S. mansoni* infection, however, eggs may be carried from the portal area through the anastomatic veins as a result of portal obstruction caused by hepatic cirrhosis; the eggs are then carried to the right side of the heart and thus to the lungs. In these cases if the infection is severe, repeated massive infection of the lung occurs and is accompanied by marked diffuse vascular lesions, causing a typical Ayerza's syndrome which is usually associated with hepatosplenomegaly.

It should be added, however, that since some of the cases of hepatosplenomegaly are associated with *S. haematobium* infection of the large intestine, eggs of *haematobium* may well be found in the sputum in some cases of Ayerza's disease due to schistosome infection. Actually in the last few years we have found that a high percentage of cases of bilharzial dysentery are due to *haematobium* infection.

Gelfand (1950) has reported observations in South Africa which accord with

our findings. Among 33 cases of *S. mansoni* infection, only 4 exhibited the lateral spined eggs of this species in the lungs after digestion of the lung, but terminal spined eggs were found in the lungs of 60 out of 99 cases of *S. haematobium* infection. Of 13 cases of schistosomal cirrhosis, 4 were found to have terminal spined eggs in the sputum.

SUMMARY

Examination of the sputum of a group of 62 patients suffering from bilharziasis revealed schistosome eggs in 22 patients. In 14 of these cases the patients were suffering from hepatosplenomegaly, and radiological examination of their chests was suggestive of bilharziasis. The sputum should be examined on at least six consecutive days to discover such cases. All cases of hemoptysis in bilharziasis patients should be examined to exclude pulmonary infection due to schistosomes.

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SUGGESTIONS CONCERNING AN INDEX OF EXPERIMENTAL FILARIA INFECTION IN MOSQUITOES[1]

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INTRODUCTION

The role that a mosquito species plays in the transmission of a filaria parasite is dependent upon both the bio-ecological characteristics of the species and its susceptibility to infection with the parasite. Generally, it has been difficult to assess fully the contributions that the various expressions of the bio-ecological characteristics make toward the overall effectiveness of a species as a possible vector. On the other hand, evidence relating to susceptibility of the mosquito is fairly direct, and thus the index of experimental infection probably has received the most widespread attention. Another measure, the index of natural infection, has been employed but on a rather limited scale due to such relatively uninvestigated problems as the identification of filaria larvae in natural infections.

An appropriate index of experimental infection, although incapable of resolving the various problems associated with filaria transmission, could nevertheless provide a useful means of exposing mutual responses of the mosquito and the filaria larva. Such information would aid considerably in a more critical evaluation of suspected vectors of human and animal filariae.

The prevalent method of arriving at an index of experimental infection has been to feed mosquitoes upon a definitive host with a demonstrable microfilaremia, allow sufficient time for the extrinsic incubation period, and then to dissect the mosquitoes and ascertain the percentage of individuals harboring larval stages of the parasite. This has been referred to as the "total infectibility percentage" (Newton, Wright and Pratt, 1945; Eyles and Most, 1947). These authors based the percentages upon the presence of fully matured larvae. Conclusions drawn from such percentage infection rates, together with inferences based upon distribution and biting habits, quite often contain suggestions that particular species of mosquitoes may or may not represent potential vectors in a given region.

Perhaps one of the first attempts to arrive at a more dynamic analysis of experimental infection was made by Brug (1939) during investigations of possible mosquito hosts of *Wuchereria bancrofti* and *W. malayi* in the Celebes. He presented diagrams to show at a glance the general reaction of the mosquito to the parasite throughout the period of extrinsic incubation. Recent work by Bertram (1949, 1950) on *Litomosoides carinii* has provided a more quantitative background

[1] The writer is especially indebted to Dr. Walter L. Newton, Laboratory of Tropical Diseases, National Microbiological Institute, whose helpful criticisms and valuable suggestions were a constant source of stimulation. Acknowledgment also is made for the suggestions of Mr. Marvin Schneiderman, Biometrics Section, National Cancer Institute, who reviewed this paper.

to the problem by a brilliant exposition of the relations between the parasite and its invertebrate host, *Bdellonyssus bacoti*. With the mite-borne filaria of the cotton rat it has been possible to evaluate actual vector efficiency because of the parasite's relatively short life cycle, the specificity of the invertebrate host, and the ease of maintaining both cotton rats and mites in the laboratory. Thus far opportunities for similar studies with a mosquito-borne filaria parasite have not developed.

THE CONCEPT OF HOST EFFICIENCY

In recent work with *Dirofilaria immitis* (Kartman, 1953a) it was suggested that an index of experimental infection might be based in part upon a host efficiency ratio which expressed numerically daily relations between mosquito and parasite. Host efficiency was derived in the following way: immediately after a batch of mosquitoes had been fed upon an infected dog a certain number of individuals were dissected for the purpose of determining the average number of microfilariae ingested per female. Subsequently, females from the same batch were dissected each day during a twenty day period, and the number of developing larvae was recorded. The ratio of the total number of developing worms recovered during this period to the number of microfilariae theoretically ingested was designated as the host efficiency ratio. The total number of infective larvae also was used to obtain a ratio called the infective potential. Comparisons of the host efficiencies among several species of mosquitoes were made after they had fed upon the same infected host and were maintained under identical laboratory conditions.

The above concept of host efficiency was an attempt to obtain a dynamic evaluation of the host-parasite responses throughout a substantial portion of the period of extrinsic incubation of the parasite. It was thought that a cumulative total of developing filaria larvae, based upon daily mosquito dissections, would reflect to some extent the ability of the parasite to develop in the mosquito from day to day.

Further evaluation of the concept of host efficiency suggests that for purposes of comparing several species it might better be based solely upon third stage larvae counted at the end of a period arbitrarily selected as one in which infective larvae are known to develop in one or more of the mosquito species. This would avoid confusion attending instances in which the filariae may develop beyond the microfilarial stage but not as far as the third stage. As an example, suppose species "A" and "B" ingested an average of 40 microfilariae each and, at the end of 15 days, each had 20 developing larvae. However "A" had mostly third stage larvae whereas most of the larvae in "B" were "sausage" types. Obviously, both host species should not have the same efficiency ratios, yet a ratio based only on developing forms would give equal host efficiencies. Possibly the attempt to combine into a single formula an evaluation of early as well as late host-parasite relationships introduces complications which render the host efficiency ratio less useful as a numerical symbol. Accordingly, we may modify the original expression as follows:

$$\text{Host efficiency} = \frac{\text{mean no. 3rd stage larvae per surviving mosquito}}{\text{mean no. mf. per mosquito in samples shortly after feeding}}$$

It should be mentioned that the mean number of microfilariae ingested per mosquito is a value which is undoubtedly subject to a good deal of sample variation. It seems essential that this variation be known in computing an efficiency ratio. For example, small batches of *Aedes aegypti* which were fed upon an infected dog approximately during the same hour showed the following:

No. females	*Mean mf. ingested*
19	18.4
22	24.5
17	27.8
26	37.8
21	30.1
14	23.6
20	19.0
20	32.4
20	41.0

Obviously, the use of any one of the above batches to determine the figure for average number of microfilariae ingested for all of the other batches combined would not be valid. Thus it is important that sufficient numbers of mosquitoes be used since a large variation would require many early dissections. The use of a vertebrate host with low microfilaremia, or *in vitro* feeding techniques might reduce the degree of variance in numbers of ingested microfilariae (Kartman, 1953a).

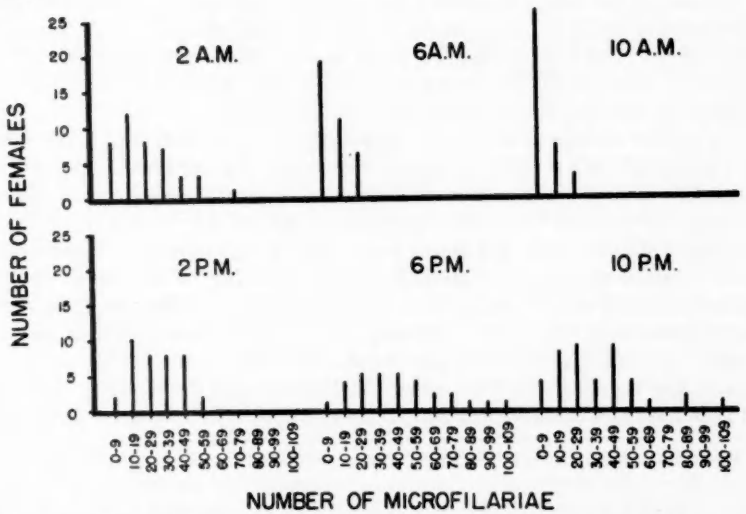

FIG. 1. Frequency distributions of *Aedes aegypti* exhibiting varying numbers of *Dirofilaria immitis* microfilariae in midguts at 4-hour interval feedings (from data in Kartman, 1953a).

Mosquito feedings at different times upon a filaria parasite exhibiting periodicity would produce very considerable variation and should be avoided. This can be seen in Figure 1 which shows the frequencies of mosquitoes with varying numbers of ingested microfilariae as influenced by time of feeding.

INDEX OF EXPERIMENTAL INFECTION

An index of experimental infection would approach the ideal if it were a numerical expression of the resultant of the significant host and parasite reactions in a given experimental situation. However it is suggested that an index should be based upon easily derived components juxtaposed in a simple formula useful for the simultaneous comparison of the susceptibility of several species of mosquitoes. With these principles in view, additional components have been related to the host efficiency ratio in an attempt to arrive at a more valid index. These are of the categories described below.

Survival rate of the host

Essentially, this is the percentage of engorged female mosquitoes surviving the period of extrinsic incubation of the parasite. The period of extrinsic incubation is the number of days required for development of infective (third) stage larvae. If several host species are tested, a period should be chosen which allows sufficient time for infective larvae to develop in some of the species in which development ordinarily occurs. For example, the following mosquitoes were infected with *Dirofilaria immitis* which required the indicated minimum periods of incubation to attain infective stages (from Kartman, 1953a): *Anopheles quadrimaculatus*, 12; *A. freeborni*, 12; *Culex quinquefasciatus*, 13; *C. pipiens*, 10; *Aedes aegypti*, 14; *A. albopictus*, 12. Since the average incubation period of the parasite in these mosquitoes was about 12 days, the period for computation of the survival rate may arbitrarily be set at 15 days.

It is known that some mosquito species survive laboratory conditions better than others, but the possible functional relation of the survival rate of infected mosquitoes to that of an uninfected series has not been investigated.[2] Although the mortality pattern of mosquitoes appears generally to follow a geometric progression (Bates, 1949), the actual death rate of a given species would appear to be a specific problem in experiments that introduce factors which may influence the death rate. Thus, in comparing several hosts, differences in mortality after exposure may or may not represent clear cut differences in tolerance to the parasite. Accordingly, consideration should be given to the use of uninfected controls the significance of whose survival rate would be tested statistically and then used as a basis for evaluating survival of a series infected with filariae.

[2] While the present paper was in press, a report was noted which presents much needed information on the problem of the survival of filaria-infected mosquitoes under experimental conditions (Kershaw, W. E., M. M. J. Lavoipierre and Chalmers, T. A., 1953. Studies on the intake of microfilariae by their insect vectors, their survival, and their effect on the survival of their vectors I. *Dirofilaria immitis* and *Aedes aegypti*, *Ann. Trop. Med. Parasit.* **47**: 207-224.)

Infection rate of the host

In the first place, a distinction must be made between mosquitoes harboring undeveloped microfilariae and those with developed larvae at the end of the incubation period. The first may be called positive while the latter may be thought of as infected. Secondly, there should be consistency in whether the infection rate is based upon the number of mosquitoes with any developing larvae or those exclusively with third stage larvae at the termination of incubation. In many instances, infection rates have been based upon the percentage of females with larvae in any stage of development; often, individuals harboring only microfilariae have been considered infected. We have already indicated that the host efficiency ratio should be based upon third stage larvae only and it would seem that restriction to this stage would similarly provide a more consistent and meaningful infection rate. Accordingly, the derivation of this component is suggested as follows:

$$\text{Infection rate} = \frac{\text{no. mosquitoes with 3rd stage larvae at end of incubation}}{\text{no. surviving mosquitoes at end of incubation}}$$

It is suggested that the appropriate effects of the three components may be reflected by an index relating these components in the following manner:

$$\text{Index of experimental infection} = (\text{survival rate}) \times (\text{infection rate}) \times (\text{efficiency ratio})$$

DATA AND DISCUSSION

Application of the formula indicated above to data derived from experiments with *Dirofilaria immitis* and various species of mosquitoes has been attempted. These data are taken from work previously recorded (Kartman, 1953a and b) and are supplemented by additional unpublished data. For an account of the materials and methods used in the infection experiments the reader is referred to the report by Kartman (1953a). It should be pointed out that these experiments were carried out in an air-conditioned insectary with the temperature maintained constantly at approximately 27°C. and with 80 to 90 per cent relative humidity. All feedings were made on the same infected dog (unless otherwise indicated) and at approximately the same time. Obviously, marked variations in temperature and humidity could vary the time required for larval development and thus effect the components used to derive the index of experimental infection. It is to be emphasized that the data used here are presented as examples rather than as definitive material since they were gathered in another connection.

Table 1 presents the indices of experimental infection for several species of mosquitoes tested as intermediate hosts for the dog heartworm, *Dirofilaria immitis*. These indices appear adequately to differentiate between good, fair, and poor hosts and it would not be difficult to select one of the species for further work with the particular degree of susceptibility desired. If susceptibility tests with other species of mosquitoes were planned with the same strain of the para-

TABLE 1

The index of experimental infection of various species of mosquitoes infected with Dirofilaria immitis *(based upon a 15 day incubation period)*

HOST SPECIES	NUMBER FEMALES USED	SURVIVAL RATE	INFECTION RATE	EFFICIENCY RATIO	INDEX EXP. INF.
Anopheles quadrimaculatus	445	0.47	0.99	0.50	0.23
A. freeborni	431	0.46	0.90	0.23	0.09
Culex pipiens	301	0.66	0.29	0.006	0.001
C. quinquefasciatus	322	0.65	0.40	0.02	0.005
Aedes aegypti	358	0.44	0.10	0.03	0.001
A. albopictus	314	0.63	0.99	0.60	0.37

site, either the *Anopheles quadrimaculatus* or *Aedes albopictus* strains used here could be employed as the standard of reference.

An important requirement in this type of comparative test is some estimate of the variability of an index for a given species under the same conditions. Thus if species "A" had an index of 0.4 and species "B" an index of 0.2 it would be essential to know whether such differences are statistically significant. Thus it would be important to perform replications of the tests to determine the variation in the index for a given species. In this connection it is of interest to compare the indices of *A. aegypti* in Table 1 with the United States strain and the controls in Table 2. All of these *aegypti* were from the same original laboratory strain and were tested at different times in connection with different experiments, but approximately under identical atmospheric conditions and with the same vertebrate host. The similarity of their indices is striking and the only component which varied noticeably was the survival rate. A similar approximation of indices is attained by the *A. quadrimaculatus* in Table 1 and other females from the same strain shown in the top line of Table 3.

In Table 2 the indices have been ascertained for several strains of *A. aegypti*

TABLE 2

The index of experimental infection of geographically isolated, and artificially selected, strains of Aedes aegypti *infected with* Dirofilaria immitis *(based upon a 15 day incubation period)*

HOST STRAIN	NUMBER FEMALES USED	SURVIVAL RATE	INFECTION RATE	EFFICIENCY RATIO	INDEX EXP. INF.
United States	149	0.56	0.10	0.03	0.001
Hawaii	153	0.61	0.15	0.11	0.01
South Africa	141	0.62	0.06	0.03	0.001
Anglo-Egyptian Sudan	112	0.71	0.07	0.08	0.003
Fiji	109	0.47	0.01	0.003	0.00001
No. 30—susceptible	168	0.35	0.25	0.24	0.02
No. 67—susceptible	218	0.28	0.33	0.18	0.01
No. 41—refractory	194	0.38	0.04	0.007	0.0001
No. 87—refractory	186	0.42	0	0	0
Controls	263	0.35	0.11	0.03	0.001

TABLE 3

Effect upon the index of experimental infection of feeding Anopheles quadrimaculatus *upon hosts with different* Dirofilaria immitis *microfilaremias (based upon a 15 day incubation period)*

HOST NO.	MF./CM³	NUMBER FEMALES USED	SURVIVAL RATE	INFECTION RATE	EFFICIENCY RATIO	INDEX OF EXPERIMENTAL INFECTION
1	16,000–18,000	105	0.47	0.90	0.44	0.18
2	30,000–34,000	900	0.05	0.20 (1.00)*	0.005 (0.64)*	0.0005 (0.03)*

* Based upon 2nd stage larvae only.

from geographically isolated regions, and for artificially selected strains of the same species. These indices not only substantiate previous observations regarding the strains (Kartman, 1953a) but emphasize the differences between the susceptible and refractory strains with a greater degree of sensitivity and uniformity. In this case, the inclusion of a highly susceptible species as a comparative standard would have indicated that the Hawaiian *aegypti* and the artificially selected susceptible strains, although better hosts than the other strains, were nevertheless rather poor hosts.

The case shown in Table 3 has been selected to emphasize the necessity of feeding all species of mosquitoes upon the same infected vertebrate host if comparable results are to be achieved. Differences both in microfilaremia and in parasite strains undoubtedly would affect development in the mosquito. In the present example it is assumed that the parasite strains are the same since both infected dogs came from the same region (Kartman, 1953b). The main effect of the high microfilaremia in dog no. 2 was to produce an enormous mosquito mortality as shown by the low survival rate. The other components also were depressed because development of the larvae in the surviving individuals was retarded and very few 3rd stage larvae were in evidence at 15 days. Thus the index is exceedingly small when compared with that in the mosquitoes from the same batch fed on dog no. 1.

If a supplementary index, based upon second stage *D. immitis* larvae, is derived for the mosquitoes fed upon dog no. 2, the figures shown in parenthesis in Table 3 are the result. In this case the index has been increased, but it still does not compare with that of the first group of mosquitoes inasmuch as the survival rate cannot be changed. Thus these indices appear to symbolize a fundamentally unbalanced host-parasite relation.

The index suggested here primarily has been formulated to provide an expression of overall susceptibility of a mosquito species to a filarial parasite and employs the third stage filarial larva as the basis for derivation of all components making up the index. On the other hand, if it is desired to evaluate and compare early development, a shorter time period could be set, e.g., 5 days, at the end of which time some of the mosquitoes could be examined. The infection rate would be the

proportion of survivors harboring larval stages normally found by 5 days in a susceptible host species used as a standard. The survival rate would be the proportion of infected mosquitoes surviving 5 days. The efficiency ratio would have the average number of 5-day type larvae in the numerator, and the denominator would be the same as already given above. Thus species "A" and "B" could have similar indices at 5 days, but at 15 days have different indices because the larvae developed to the third stage in one and not in the other.

It seems clear that many problems concerning host-parasite relations remain to be solved before an index of experimental infection which is entirely valid and complete may be formulated. It is suggested that, whatever type of index may be developed, it should avoid involved formulations which may tend to diminish its usefulness. Thus an expression which attempts to evaluate simultaneously the factors which have already been demonstrated to affect the likelihood that a given species will be a good host would appear to be of practical value.

The index, as here suggested, is subject to variation. In order to compare indices for different species, the amount of this variation should be known. Statistical studies will have to be conducted to find how large this variation is, so that one may be able to know how much confidence can be placed in an experimentally determined index.

It should be emphasized that, at best, an index of experimental infection could indicate only a part of the theoretical role that a given mosquito species may play in the epidemiology or epizootiology of filariasis. As in the study of the susceptibility of anopheline mosquitoes to malaria infection, ready infectibility in the laboratory is not necessarily proof that a species is an important transmitter under natural conditions. Thus Boyd (1949), in a discussion of these problems as related to malaria, points out that many questions regarding the ecology and physiology of the mosquito host must be resolved before the question of the dangerousness of a species is answerable.

SUMMARY

The need for a more adequate index of experimental infection in laboratory studies of mosquito hosts and filarial parasites is noted.

The concept of host efficiency is discussed and it is suggested that recent attempts to combine into a single formula an evaluation of early as well as late host-parasite relations render the host efficiency ratio less useful as a numerical symbol. Accordingly, an expression for host efficiency is proposed as a ratio of the mean number of third stage larvae per mosquito surviving at the termination of the filarial incubation period to the mean number of microfilariae per mosquito in samples shortly after feeding upon an infected vertebrate host.

Two additional components are suggested as follows: 1) the survival rate of the host, defined as the percentage of engorged female mosquitoes surviving the period of extrinsic incubation of the parasite; and 2) the infection rate of the host, derived by dividing the number of mosquitoes with third stage larvae at the termination of the parasite's incubation period by the number of surviving mosquitoes at the end of incubation.

It is suggested that the appropriate effects of the above three components may be reflected by an index relating them in the following manner:

Index of experimental infection
= (survival rate) × (infection rate) × (efficiency ratio)

The above formula is applied to experiments comparing the susceptibility of various species of mosquitoes to *Dirofilaria immitis*. On this basis, it is suggested that the present index, although subject to certain limitations, appears to be of practical value insofar as it succeeds in evaluating simultaneously certain factors which already have been demonstrated to affect the likelihood that a given species of mosquito will be a good host for a filarial parasite.

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PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA

IV. Hemoglobin Values and their Relation to the Intensity of Hookworm Infections in the Labor Force

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Anemia, as manifested by low hemoglobin content of the blood, is one of the commonest clinical findings among Indians as a whole, and in this regard native workers in industry are by no means excepted. Malnutrition, malaria, early and repeated pregnancies, and hookworm infection, alone or in combination, are generally regarded as the major underlying factors in these anemias. In the present report, however, only the last of these factors is considered in relation to hemoglobin values, the data having been collected in conjunction with studies of intestinal parasitic infections among 750 mill workers, their supervisors, and the latter's servants, at the Ludlow Jute Company's mill near Chengail, Howrah District, West Bengal (Chernin, 1953a, b).

MATERIALS AND METHODS

The studies among the mill workers were conducted between March 4 and June 23, 1952, among the supervisors between March 26 and July 2, 1952, and among the servants from June 25 to July 4, 1952, at the laboratory of the Ludlow dispensary-hospital. All surveys were undertaken and completed during the non-malaria season. Hemoglobin determinations were made between 8:00 and 9:00 A.M. daily using a Spencer hemoglobinometer, an instrument which permits direct readings of hemoglobin in terms of grams per 100 ml. of blood. The hemoglobinometer was checked for accuracy against parallel data obtained by the Hellige method before the survey was begun. Finger blood obtained by needle puncture was used exclusively and each determination represented an average (to the nearest one-half gram) of two readings per sample.

The general technics of stool examination have already been described (Chernin, 1954a), but for purposes of determining the intensity of the hookworm infections thus uncovered, the Stoll dilution egg-count method was employed. A single count of the eggs found by this technic was made for each specimen and these counts were then reduced to the basis of formed stool and expressed as eggs-per-gram. In the data to be presented below, "negative" indicates that no hookworm eggs were found on direct smears or in zinc sulfate concentration preparations. However, many infections detected by these technics were so light that no eggs were found in the Stoll dilution preparations; such "negative count" cases were recorded as falling in the 1–99 egg-count group.

The composition of the sample of 750 workers involved in this study has been detailed previously (Chernin, 1954a). Similarly, the composition of the comparison groups (supervisory staff members, their families and their servants) has

also been presented in another connection (Chernin, 1954b). For present purposes it is sufficient to note that of the 7,000 workers employed at Chengail approximately one-half live inside the compound under conditions of environmental sanitation vastly superior to those prevailing in the nearby villages where the balance of the labor force is resident.

PREVIOUS STUDIES OF HEMOGLOBIN VALUES IN INDIA

Hemoglobin values among Indians have only come under investigation in the comparatively recent past. The reports do not lend themselves to individual attention here because they were conducted in widely separated parts of India with subjects from a variety of socio-economic groups, and because different technics were employed for the determination of hemoglobin content. However, for the purposes of general comparison with the data to be presented in this report, a summary of the results of studies of paired groups of males and females in the same locales has been compiled and is presented in Table 1. Reference should also be made to the more general studies of a rural population near Calcutta by Lal and Seal (1949), to the studies of tea-garden coolies by Hare (1940), and to additional literature cited by Napier (1940).

PREVALENCE AND INTENSITY OF HOOKWORM INFECTIONS

The intestinal parasite surveys referred to earlier yielded general background information concerning the prevalence of hookworm infections among the various groups represented in the mill population and a summary of these data is presented in Table 2. The infection is somewhat more prevalent in males than females, but only slight differences are found between Hindus and Muslims or between mill-resident and village-resident employees. In all, 68.7 per cent of the mill workers harbored hookworms, a figure which is in obvious contrast with that for the supervisory personnel, and which is considerably higher than the 49 per cent rate of infection among the staff's servants. The distribution of hook-

TABLE 1

A summary of the results of past hemoglobin studies among Indian males and females in various locales. Additional references are given in the text

LOCALE AND AUTHORS	MALES			FEMALES		
	No.	Mean Hb.	Range	No.	Mean Hb.	Range
Assam (Napier and Das Gupta, 1935b)	24	11.8	7.6–15.8	17	10.0	6.9–13.3
Bombay (Sokhey et al., 1937, 1938)	121	15.4	12.8–17.7	101	13.0	10.5–16.0
Madras (Sankaran et al., 1938)	125	16.6	12.2–20.3	62	13.7	11.8–16.3
Bihar (Prasad and Chowdhury, 1943)	102	13.6	9.5–15.4	71	12.5	9.1–15.0
Coonoor* (Ramalingaswami et al., 1950)	100	15.8	13.1–20.3	—	—	—
Coonoor* (Rao, 1938)	—	—	—	100	15.8	8.0–20.5
	—	—	—	100†	15.5	8.5–21.0

* Coonoor is at 6,000 ft. altitude.

† "Normal" pregnant females.

TABLE 2

Summary of data relative to the prevalence of hookworm infections among Ludlow mill workers, supervisory staff, and staff servants. (Taken from Chernin, 1954a, b)

GROUPS UNDER STUDY	NO. EXAMINED	INFECTED	
		No.	Per cent
Males	645	454	70.4
Females	105	61	58.1
Hindus	566	377	66.7
Muslims	184	138	75.0
Mill residents	384	259	67.4
Village residents	366	256	69.9
All mill workers	750	515	68.7
Supervisory staff	62	0	—
Staff servants	47	23	48.9

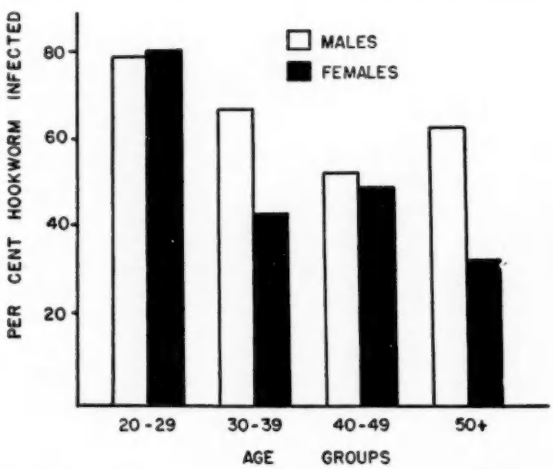

FIG. 1. The prevalence of hookworm infection in relation to age and sex among 750 workers at the Ludlow mill.

worm infections among the 750 workers (by sex and age) is illustrated in Figure 1. The infection is most prevalent in the youngest age group (20–29), but with increase in age there is an irregular decrease in the prevalence of infection. The rates for males above age 30 are always somewhat in excess of those for females in corresponding age groups, although the infection is prevalent to the same degree in both sexes in the age group 20–29.

Figure 2 represents the distribution of male and female mill workers in the

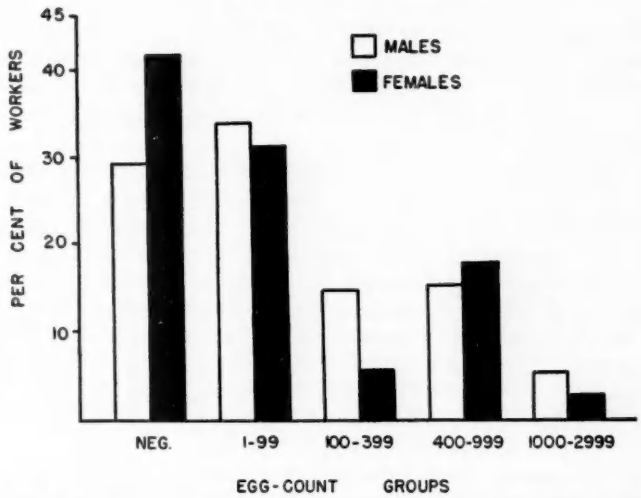

FIG. 2. The relative proportions of male and female Ludlow mill workers in the various hookworm egg-count groups.

several hookworm egg-count groups and serves to illustrate that the infections in this population are, on the whole, rather light ones. In fact, only about 20 per cent fall into that range which is usually considered to be of "possible" clinical significance (an isolated case with an egg count of 19,400 per gram is not included in the figure). It is also of interest to note here that relatively similar proportions of males and females are found in most of the egg-count groups. The data in Table 3 indicate that there are no consistently significant changes in the intensity of the infection with increased age among males, but among females it appears that the infections tend to decrease in intensity with increased

TABLE 3

The relative proportions by age of male and female Ludlow mill workers in the various hookworm egg-count groups

HOOKWORM EGG-PER-GRAM GROUPS	AGE GROUPS							
	Males				Females			
	20–29 (326)	30–39 (152)	40–49 (120)	50+ (47)	20–29 (41)	30–39 (46)	40–49 (12)	50+ (6)
Negative	21.2	32.2	46.6	36.2	19.5	56.5	50.0	66.7
1–99	36.8	36.8	21.7	38.3	46.3	15.5	41.7	33.3
100–399	15.6	12.5	17.5	10.6	9.8	4.4	0.0	0.0
400–999	19.6	13.8	10.0	10.6	19.5	21.7	8.3	0.0
1,000–2,999	6.8	4.0	4.2	4.3	4.9	2.2	0.0	0.0
3,000–9,999	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10,000+	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0

age. No marked differences were noted between Hindus and Muslims with regard to intensity of hookworm infection and similarly, no differences appeared to exist between mill- and village-resident employees in this respect, despite the fact that the sanitary conditions under which these two groups live are very different.

Seven male workers with the heaviest hookworm infections were treated with tetrachlorethylene and the worms recovered from all the stools passed within 8 hours of treatment. Of the 706 worms thus taken, 87 per cent (281 males and 332 females) were identified as *Necator americanus*, and 13 per cent (42 males and 51 females) as *Ancylostoma duodenale*. One of the seven workers had *N. americanus* alone, 2 had only *A. duodenale*, and 4 had both species of worm in varying proportions. These figures complement those of Chandler (1928) who reported that 80 per cent of the hookworms in Bengal were necators and the remainder ancylostomes.

HEMOGLOBIN VALUES

The relative proportions of male and female mill workers in the designated hemoglobin groups are shown in Figure 3. It is plainly evident that a large proportion of the females (over 12 per cent) fall into an exceedingly low hemoglobin group (5.0–7.5 gm./100 ml.) and that the females as a whole are very poorly represented in the higher hemoglobin categories. The values for the males, on

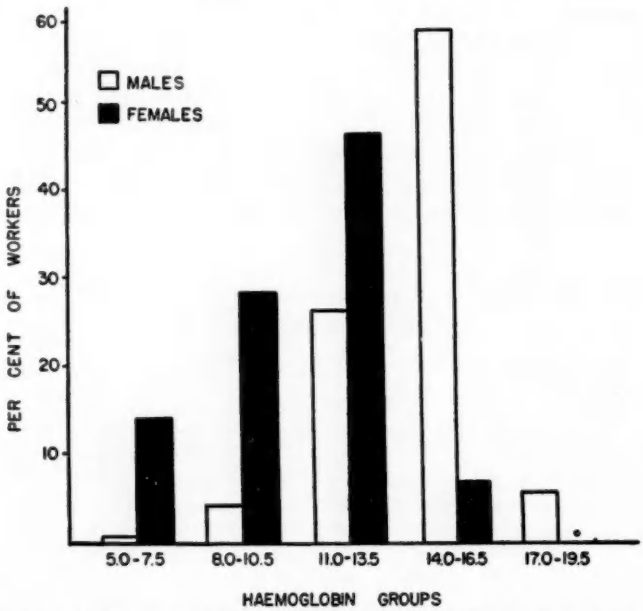

FIG. 3. The relative proportions of male and female Ludlow mill workers in various hemoglobin groups. Hemoglobin is expressed in gm./100 ml. of blood.

TABLE 4

Mean hemoglobin values (in gm./100 ml. of blood) for Ludlow mill workers by sex, religion, and residence, and comparison with hemoglobin values for members of the supervisory staff and their servants

GROUPS UNDER STUDY	NO. EXAMINED	MEAN Hb. AND STANDARD DEVIATION
All male workers	645	14.3 ± 1.7 (Range 6.0–19.0)
All female workers	105	10.8 ± 2.1 (Range 5.5–16.0)
Male Hindus	465	14.4 ± 1.7
Male Muslims	180	14.2 ± 1.6
Male mill residents	286	14.5 ± 1.9
Male village residents	359	14.2 ± 1.6
Staff males	32	16.8 ± 0.9 (Range 15.0–19.5)
Staff females	17	14.3 ± 1.1 (Range 12.5–16.5)
Male servants	39	15.0 ± 1.1 (Range 12.0–18.5)
Female servants	8	14.0 ± 1.3 (Range 11.5–16.0)

the other hand, are well clustered in and about the hemoglobin group 14.0–16.5 gm./100 ml., although a scattering appear at either end of the scale.

The mean hemoglobin values for male and female mill workers are shown in Table 4. Analysis indicated that for each sex the mean hemoglobin values did not vary significantly with age. However, there are marked and statistically significant[1] differences between the mean hemoglobin values for males and for females at all age levels from 20 to 50-plus, and these differences are also reflected in the totals for the two sexes as shown in Table 4. A sharp contrast with these data is provided by the results of the hemoglobin studies conducted among 49 adults of the supervisory group and their 47 household servants (the supervisory group is composed one-third of Indians and two-thirds of British and Americans of similar socio-economic status; further details pertaining to this and to the servant group have been published previously). The data for these groups (Table 4) indicate that the servants fall into a position somewhat between the supervisors and the mill workers with regard to mean hemoglobin levels; thus, the respective mean values for male and female mill workers are significantly lower than the corresponding means for the servants or the supervisory group.

Data comparing religious and residence groups among the workers are pre-

[1] An observed difference is taken to be significant statistically if the difference in means divided by the standard error of the difference yields a value of 2 or more.

TABLE 5

Mean hemoglobin values (in gm./100 ml. of blood) in relation to the degree of hookworm infection among male and female mill workers at Ludlow

HOOKWORM EGG-PER-GRAM GROUP	MALES (645)		FEMALES (105)	
	Number	Mean Hb.	Number	Mean Hb.
1–99	220	14.22	33	10.71
100–399	96	14.33	6	11.50
400–999	102	15.09	19	9.63
1000–2999	35	13.39	3	8.33
3000–9999	0	—	0	—
10000+	1	16.0	0	—
All hookworm positives	454	14.38	61	10.34
All hookworm negatives	191	14.16	44	11.35

sented in Table 4 (the data for female mill workers are not analyzed separately by religion and residence since nearly all were Hindus and these, with rare exception, lived within the compound). Analysis indicates that there is no significant difference in the mean hemoglobin values of Hindus and Muslims, and that only a minor difference exists in the mean hemoglobin content between mill- and village-resident employees.

HEMOGLOBIN VALUES AND THE INTENSITY OF HOOKWORM INFECTION

The mean hemoglobin values for male and female mill workers in each of the hookworm egg-count groups are presented in Table 5. No consistent decrease in mean hemoglobin values occurs among the males with increased intensity of hookworm infections as measured by the egg-counts. Furthermore, the mean hemoglobin value for all hookworm-positives is slightly *higher* than the mean for those with no hookworm infection. The females, however, present an entirely different picture: the mean hemoglobin values for females in the two highest egg-count groups (taken together) are significantly lower than the means corresponding to the less intense infections; in addition, the mean hemoglobin value for all the female hookworm-positives (10.34 gm./100 ml.) is significantly lower than the mean for the females who were free of the infection (11.35 gm./100 ml.).

DISCUSSION

The prevalence of hookworm infections among Ludlow mill workers is of the same general order as that established by Chandler's studies for Bengal as a whole (Chandler, 1926). Indeed, a 1919 Report on Sanitation in Bengal (not seen in the original but cited by Chandler) gives the prevalence of hookworm infection among "coolies in a mill area near Calcutta" as 70 per cent, a figure which closely agrees with the 68.7 per cent rate of hookworm prevalence disclosed by the more recent survey (Chernin, 1954a). The distribution of the mill workers in the various egg-count groups (Fig. 2) also substantiates Chandler's general observations

in illustrating that most of the hookworm infections in this area are not intense ones by ordinary standards. Chandler (1928) concluded that egg-counts of 500 or less may be ignored even when they occur in children and that persons in his 600–2,000 egg-per-gram group might be considered as borderline clinical cases who suffer very little unless undernourished, overworked, or suffering from chronic diseases. In the present study, only a relatively small proportion fall into this borderline group.

The mean hemoglobin value established for the 645 male mill workers (14.31 gm./100 ml.) corresponds best with the mean value of 14.15 gm./100 ml. cited by Napier and Das Gupta (1935a) for a small group of 16 Calcutta menials, while the mean for the 105 female mill workers (10.76 gm./100 ml.) is in closest agreement with the values given for normal pregnant and nonpregnant Assamese tea-garden coolies (Napier and Billimoria, 1937). It is noteworthy of the present data, however, that the minimum hemoglobin values recorded for both males and females are lower than the minima in any other survey cited. The values for the supervisory group fall well within the hemoglobin range usually accepted as normal in the United States, i.e. 14–18 gm./100 ml. for males and 12–16 gm./100 ml. for females. The striking differences in mean hemoglobin values between the body of mill workers and their Indo-European supervisors, particularly in the case of the females, hardly requires additional comment, except that these differences may perhaps be taken as but one manifestation of the huge socio-economic gap that separates the bulk of Indians from most Westerners.

It appears from the data in Table 5 that the male mill workers are, for the most part, able to compensate for any blood loss due to hookworm infection at those intensity levels at which it occurs among them. Only a single extremely heavy case of hookworm infection was encountered among the males, and this in a 30-year old native of West Bengal whose egg-count was 19,400 per gram and whose hemoglobin was 16.0 gm./100 ml. Since the case was a most unusual one, both examinations were repeated and similar values were again obtained. The individual appeared well nourished, no complaints or symptoms suggestive of severe hookworm infection were presented, and a physical examination revealed nothing remarkable.

The female mill workers, by contrast with the males, do not seem to be able to compensate for blood loss when the intensity of infection exceeds 400 eggs-per-gram. These individuals fall into the category established by Chandler (*vide supra*) of those who suffer at this level of hookworm infection when concomitants such as malnourishment, overwork, and chronic diseases (such as malaria) are also operative. Although these have not been the subject of detailed study among the female employees of the mill, general observations made over the period of a year leave little doubt but that all are of some significance in this context. The further problems raised by early and repeated pregnancies should also be considered along with the more obvious factors mentioned above.

Although hookworm infection does not appear to be the over-riding public health problem that the prevalence data alone might suggest, it is nevertheless of

significance at least insofar as some of the female mill workers are concerned. From a broader viewpoint, however, it should be evident that anemia among the workers (especially among the females) would still present a serious medical and public health problem even in the complete absence of hookworm infection. This problem must be met, particularly if efficiency in industrial operation is a prime consideration.

SUMMARY

A study of hemoglobin values and their relationship to hookworm infections was conducted among workers, supervisors and servants at the Ludlow Jute Company mill, Chengail, Howrah District, West Bengal. Although nearly 70 per cent of the mill workers were infected with hookworm, the infections were very light. Mean hemoglobin values for the mill workers ranged considerably below those of the supervisory group. Hookworm infection did not appear to influence significantly the hemoglobin values for male mill workers; among the females, however, hemoglobin values decreased with increased degrees of hookworm infection, compensation failing when infections exceeded 400 eggs per gram.

Hookworm infections did not appear to present a serious public health problem among the mill workers (except for the more heavily parasitized females). However, anemia, especially in the females would remain a serious problem even in the complete absence of hookworm infection.

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PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA

V. Eosinophile Levels and their Relation to Intestinal Helminthiasis in the Labor Force

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Despite the growing interest in India in certain clinical syndromes characterized by high eosinophilia, very few studies have been concerned with the determination of eosinophile levels in the Indian population at large, and even fewer have dealt with the relationship of eosinophilia to helminthiasis. An extension of information along these lines should help in establishing a better basis for evaluating the significance, and possibly even the causal factors, of eosinophilia as it occurs in India.

This report presents the results of differential white cell counts done in conjunction with studies of intestinal parasites (Chernin, 1954a, b) among workers, supervisors and servants at the mill of the Ludlow Jute Company at Chengail, Howrah District, West Bengal.

MATERIALS AND METHODS

Blood smears were made between 8 and 9 A.M. daily at the laboratory of the Ludlow hospital-dispensary. Films were air-dried, fixed in absolute methyl alcohol, stained by the Giemsa technic, and examined under oil immersion. Differential counts were based on the examination of 100 leucocytes per slide; a larger sample of cells, while desirable, was not practicable because of the volume of other work in progress.

The survey of the mill workers (a sample of 750 drawn from a worker population of 6–7,000) was conducted between March 4 and June 23, that among 51 adults of the Indo-European supervisory staff from March 26 to July 2, and among 47 of the staff's servants from June 25 to July 4, 1952. These surveys were undertaken and completed during the non-malaria season. Descriptions of the environment, information relevant to the composition of the population groups, and the technics and results of the surveys of intestinal parasitic infections have been published (Chernin, *loc. cit.*). A further account dealt in detail with the prevalence, intensity and public health significance of hookworm infections among the mill workers (Chernin, 1954c). Pertinent data from these studies will be referred to later in this report.

PREVIOUS STUDIES OF EOSINOPHILE LEVELS IN INDIANS

For purposes of comparison with the data developed during the present study it is of interest to consider briefly the results of several earlier surveys of eosinophile counts among different groups of Indians. Napier and Das Gupta (1935a) reported a mean eosinophile percentage of 6.91 (range, 0–18 per cent) among 50 normal male adults (doctors, students, and menials) in Calcutta, and

concluded on this basis that ". . . 7 per cent must be accepted as a normal eosinophile count for healthy individuals in this country." The same authors (1935b), in hematological studies of Assamese tea-garden coolies, found the mean eosinophile percentage of 24 males was 13.76 (range, 2–37), and that of 17 females was 13.74 (range, 2–43). In 1941, Napier *et al.* derived a mean eosinophile percentage of 5.6 for 123 women (doctors, students, etc.) in Calcutta, while Ramalingaswami *et al.* (1950) reported 4.4 per cent as the mean eosinophile level in a healthy group of 53 male doctors, students, and soldiers at Coonoor. Over 40 per cent of 564 university students at Colombo were found to have eosinophile counts in excess of 400 per cmm., approximately 5 per cent (Bibile *et al.*, 1950); after a study of the possible underlying factors, these authors expressed the opinion that ". . . the commonest cause of symptomless eosinophilia is an infection or infestation with metazoan parasites."

RESULTS OF DIFFERENTIAL COUNTS

Table 1 presents the detailed results of differential white cell counts and compares the mill workers with their supervisors and with the latter's servants in this regard. It is obvious that the mill workers had a much higher mean eosinophile level than did the supervisors and that the mean percentage for the servants fell somewhat between the other two. Since the ordinary range for eosinophiles is usually taken to be 0–4 per cent of the total count, it is apparent that of the means for the three groups only those of the supervisory staff may be considered "normal." There is no significant difference in the mean eosinophile levels for males as compared with females in either the supervisory group or their servants; however, a significant difference is manifest in the

TABLE 1

The results of differential white cell counts (mean percentages and their range) among mill workers, supervisors and staff servants at the mill of the Ludlow Jute Company, Chengail, India

WHITE CELLS	MILL WORKERS		SUPERVISORY STAFF		STAFF SERVANTS	
	Males (645)	Females (105)	Males (34)	Females (17)	Males (39)	Females (8)
Polymorphs	50.9	53.6	53.0	56.6	52.2	53.5
Range	14–84	15–76	35–74	42–78	24–73	37–79
Lymphocytes	27.7	28.7	27.2	30.6	30.5	33.0
Range	6–59	8–62	9–47	18–52	12–55	15–52
Monocytes	10.8	8.9	16.6	9.9	9.0	6.5
Range	0–64	1–46	0–40	0–22	2–28	4–11
Eosinophiles*	10.5 ± 7.4	8.8 ± 6.3	3.0 ± 2.0	2.9 ± 2.1	8.3 ± 2.7	6.8 ± 3.2
Range	0–58	0–27	0–8	0–9	2–22	2–12

* The standard deviation is given with each mean eosinophile value. Means are considered to differ significantly if the difference between them divided by the standard error of the difference yields a value of 2 or greater.

TABLE 2

The percentage of mill workers, by sex and by the presence or absence of helminthic infections, in various eosinophile groups

EOS. GROUPS	MALES (645)	FEMALES (105)	ALL WORKERS (750)	HELMINTH-POSITIVE (571)	HELMINTH-NEGATIVE (179)
0–4	17.8	29.5	19.5	16.8	27.9
5–9	33.6	33.3	33.6	30.6	43.0
10–14	26.2	19.0	25.2	28.5	14.5
15–19	13.8	10.5	13.3	15.4	6.7
20–29	6.8	7.6	6.9	7.0	6.7
30–39	1.1	—	0.9	1.1	0.6
40–49	0.2	—	0.1	0.2	—
50–59	0.5	—	0.4	0.4	0.6

means of the two sexes in the mill worker group. Of secondary interest are the evidences of monocytosis exhibited in these data. This tendency is particularly marked in the case of the male supervisors whose mean monocyte percentage is twice the ordinarily accepted normal maximum of 8 per cent. No suitable explanation for the monocytosis has presented itself.

The relative percentages of male and female mill workers in the several eosinophile classes are indicated in Table 2 (columns 1–3). The lowest eosinophile group (equivalent to the "normal" range of values) contained a higher proportion of females (29.5 per cent) than males (17.8 per cent). In the succeedingly higher eosinophile groups the proportion of females was either equal to or less than the proportion of males.

Mean eosinophile percentages by age and sex are shown for the mill workers in Table 3 (columns 1, 4, 7). These data indicate that a decline in eosinophile level occurs in both sexes with increased age. This tendency to decline is less marked from one age group to the next than it is if only the extremes in age groups are compared.

EOSINOPHILIA AND HELMINTHIASIS

The study of intestinal parasitic infections among the 750 mill workers (summarized in Table 4) disclosed that 571 had one or more worm infections. The proportions of helminth infected persons in the various eosinophile classes are presented in Table 2 (columns 4 and 5) along with a similar analysis based on the smaller group of helminth-free workers. These data indicate that 70 per cent of the helminth-negative persons fell into the two lowest eosinophile groups (9 per cent eosinophiles or less) while less than half of the helminth-positive workers were in the two lowest eosinophile groups. In the 2 succeeding eosinophile classes the proportion of helminth-negative persons was less than that of helminth-positives, and thereafter the classes included approximately equal proportions of both. Some 25 per cent of the helminth-infected persons and nearly 15 per cent of the helminth-negatives, had eosinophilias of 15 per cent or higher.

Mean eosinophile percentages by age, sex, and the presence or absence of

TABLE 3

Mean eosinophile percentages for male and female mill workers by age group and with regard to the presence or absence of intestinal helminthic infection. Numbers in parentheses indicate number of workers in each group

AGE GROUPS	MALES			FEMALES			TOTALS		
	Total examined (645)	Helminth-positive (503)	Helminth-negative (142)	Total examined (105)	Helminth-positive (68)	Helminth-negative (37)	Total examined (750)	Helminth-positive (571)	Helminth-negative (179)
20–29	11.8 (326)	12.2 (275)	10.1 (51)	10.0 (41)	10.9 (34)	5.4 (7)	11.6 (367)	12.0 (309)	9.5 (58)
30–39	9.4 (152)	9.6 (114)	8.6 (38)	8.7 (46)	10.2 (24)	7.1 (22)	9.2 (198)	9.7 (138)	8.1 (60)
40–49	9.6 (120)	9.2 (81)	10.5 (39)	6.3 (12)	7.5 (6)	5.2 (6)	9.3 (132)	9.1 (87)	9.8 (45)
50+	7.5 (47)	8.6 (33)	5.0 (14)	6.7 (6)	7.8 (4)	4.5 (2)	7.4 (53)	8.5 (37)	5.0 (16)
Mean and standard deviation	10.5 ±7.4	10.9 ±7.3	9.3 ±7.7	8.8 ±6.3	10.2 ±6.7	6.4 ±4.8	10.3 ±7.2	10.8 ±7.2	8.7 ±7.2

TABLE 4

The prevalence of various intestinal helminthic infections in various population groups at the Chengail mill. Data taken from Chernin, 1954a, b

HELMINTH INFECTIONS	MILL WORKERS			PER CENT 47 SERVANTS	PER CENT 61 SUPERVISORS
	Per cent 645 Males	Per cent 105 Females	Per cent 750 Total		
Hookworm sp.	70.4	58.1	68.7	49	—
Ascaris lumbricoides	10.7	2.9	9.6	17	—
Trichuris trichiura	17.7	8.6	16.4	17	2
Enterobius vermicularis	4.2	2.9	4.0	6	—
Strongyloides stercoralis	7.6	11.4	8.1	4	—
Hymenolepis nana	2.3	—	2.0	2	—
Per cent infected	77.9	64.8	76.1	79	2

intestinal helminths are shown in Table 3. Among helminth-positive males and females, considered separately or together, a general decrease in mean eosinophile percentages occurred with increased age. The mean eosinophile values fluctuate irregularly with age in the case of the helminth-negative group, but this may in part be due to the smaller number of individuals involved. The total mean eosinophile percentage was numerically higher among helminth-positives than helminth-negatives in three of the four age groups.

Table 3 (bottom row) summarizes the eosinophile data with regard to the presence or absence of intestinal helminth infections in each sex. Considering

males and females together, the mean eosinophile percentage of helminth-negatives is significantly lower than that of the helminth-positives. Similarly, among the females, the mean of the helminth-negative persons is significantly lower than that of helminth-positives. The difference in means among the males is not significant statistically; however, since further examination of the data disclosed that the mean of the helminth-negative males was abnormally raised because of some exceedingly high eosinophile counts in a very few individuals, the lack of statistical significance here is more apparent than real. A further analysis was undertaken using the technic of the chi-squared test: 235 helminth-positive and 97 helminth-negative males had eosinophile counts of 9 per cent or less, while 268 helminth-positive and 45 helminth-negative males had eosinophile percentages of 10 per cent or more. This 4-cell grouping results in a chi-squared value of 20.6, indicating that there were significantly more workers with eosinophilia of 10 per cent or more among helminth-positives than among helminth-negatives.

DISCUSSION

It is evident from the normal values exhibited by the supervisory groups (Table 1) that mere residence in the tropics is not of itself productive of eosinophilia. In fact, one-third of the supervisors were Indians who had been born and raised there, while a large proportion of the Europeans had lived in India for 5 years or longer. Of particular significance to the discussion at hand, however is the fact that only a single individual in this group was found harboring an intestinal helminthic infection (Table 4).

The eosinophilia among helminth-positive mill workers is probably more closely related to the presence of ancylostomiasis than to the other helminthiases. Hookworm infection, alone or with other helminths, was the common factor in 515 of the 571 workers in whom helminths were found (Table 4). Thus, only 56 persons (7.5 per cent of all the workers) had helminthic infections that were uncomplicated by ancylostomiasis. The mean eosinophile percentage for this group was 8.0, a value significantly lower than the mean for all cases with hookworm infection (mean, 11.07). As judged by indirect evidence, ascariasis and trichuriasis appear to be of no special consequence with regard to eosinophilia among the mill workers; although the parasite survey showed that these infections were 8 to 10 times more prevalent among Muslims than Hindus (Chernin, 1954a), the mean eosinophile percentages of male Muslims (10.3) and of male Hindus (10.6) show no corresponding differences. Eosinophilia is known to be a common accompaniment of strongyloidiasis (Hinman, 1938), but it is difficult to assess the significance of the infection in the present context because of its relatively low prevalence and because only a handful of cases occurred independently of hookworm infections. Pinworm and *Hymenolepis nana* infections are not known to be associated with an increase in eosinophiles.

The decrease in mean eosinophile levels with increased age (Table 3) is probably related in part to the relatively small but parallel decrease in hookworm prevalence in successive age groups and in part to the slightly lighter degrees of infection in the older age groups (Chernin, 1954c). Similarly, it appears

likely that the significant difference noted earlier between male and female mill workers with regard to eosinophile levels (Tables 1, 3) may be related to the greater prevalence of hookworm infections as well as to their somewhat heavier intensity among the males as compared with females. However, since hookworm infection is only one of several elements in the complex under discussion, these correlations are considered more suggestive than conclusive.

Although it has been established statistically that the mean eosinophile percentage of helminth-infected workers is significantly higher than that of helminth-free workers, it does not follow that intestinal helminthiasis must necessarily be a factor in all individuals who manifest an eosinophilia. Indeed, given a mill worker with an eosinophilia of 10–15 per cent, one could not, from this alone, state with any degree of certainty that the person did or did not have an intestinal helminthic infection. In certain areas of the world, and under circumstances where other causes of eosinophilia are relatively unimportant, it has been demonstrated that eosinophilia can be taken as an index of the prevalence of ancylostomiasis and strongyloidiasis (Liebow and Hannum, 1946). As these authors surmise, India is not such an area, for certainly causes of eosinophilia other than helminthiasis must be of consequence when more than 70 per cent of helminth-free persons have eosinophile levels in excess of 4 per cent (Table 2). Furthermore, regardless of what these causes of eosinophilia in helminth-free persons may ultimately prove to be, there is no reason to assume that they may not also be operative in persons with helminthic infections.

Because of the limited scope of this study no attempt can be made to define specifically the causes of eosinophilia (other than helminthiasis) among the mill workers. However, several observations deserve consideration inasmuch as they may help delineate areas for further investigation:

1. While eosinophilia may commonly be associated with filariasis (Strong, 1944), this infection is not heavily endemic among the workers as a group. Studies of the prevalence of filariasis (data to be published) indicate that of the 750 workers in the sample group only 41, about 5.5 per cent, might be expected to have the infection, a figure which does not go very far in explaining the eosinophilias of unknown origin.

2. It has recently been shown that persons may suffer "visceral larva migrans" resulting from accidental infection with the common ascarids of dogs and cats, and that long-sustained eosinophilia is one of the features of this condition (Beaver *et al.*, 1952). These authors suggest that invasion of man by nematode parasites of animals may be commoner than is presently evident and that "visceral larva migrans" may be the basis for some of the syndromes in which eosinophilia is of ill-defined origin. The possible significance of these observations in terms of the mill workers can only be speculated upon. However, since dogs and jackals are present in numbers in a general environment which is poor in sanitation and even poorer in sanitary concepts, it is not implausible that infections with their ascarids may occur among the workers.

3. Similarly, it is not known whether prolonged exposure to jute dust, a complication of one of the early steps in the milling process, is productive of eosinophilia, nor whether this dust contains organisms (such as mites) that might

also be inhaled. This matter may be of significance in view of the suggestions advanced by some authors that mite infestations of the respiratory tract may be responsible for bronchitis and asthma associated with high eosinophilia (Carter and d'Abrera, 1946).

4. Bronchial asthma is a common respiratory condition among the mill workers and further study may reveal that some proportion of these may actually be cases of "tropical pulmonary eosinophilia" (see Ball, 1950, for a review of this syndrome). In fact, one such case was encountered among a group of workers who were investigated further because they had been found to have very high eosinophile levels during the routine survey. This individual, a 23 year old male Hindu born in Orissa, had a long medical history of bronchitis, severe bronchitis at the time of examination, a total white cell count of 20,000, and an eosinophilia of 58 per cent. A very mild hookworm infection also was present. Shortly after the completion of a 3-week course of treatment with diethylamine acetarsol ("Acetylarsan"), the lung signs had cleared, the total leucocyte count was 7,700, and the eosinophilia had dropped to 23 per cent. Except that x-ray studies were not done, the clinical findings described in this case and the therapeutic response to arsenic all correspond to the features of "tropical pulmonary eosinophilia" as outlined by Ball (*loc. cit.*).

SUMMARY

An investigation of eosinophile levels in relation to intestinal helminthiasis among 750 workers, 51 supervisors and 47 staff servants, was conducted during March–June, 1952, at the mill of the Ludlow Jute Company, Chengail, Howrah District, West Bengal.

The mean eosinophile percentage of the mill workers was 10.3, that of the servants, 8.0, and that of the supervisors 3.0. Some 80 per cent of the mill workers had eosinophile counts in excess of 4 per cent and nearly 8 per cent of the workers had eosinophilia of 20 per cent or higher. The mean eosinophile percentage of 571 helminth-positive mill workers was 10.8, a value significantly (but not markedly) higher than the 8.7 per cent mean for 179 helminth-negative workers. The eosinophilia among helminth-positive workers appeared to be more closely related to hookworm infections than to the other helminthiases.

It is noted that eosinophile levels in individual workers cannot be taken to indicate the presence or absence of helminthic infection. Furthermore, those factors which are responsible for eosinophilia among helminth-negative workers (of whom 70 per cent had eosinophilia levels over 4 per cent) may also be expected to operate among those with intestinal helminths.

Eosinophilia among the mill workers, in relation to causes other than helminthic infections, is discussed with a view toward outlining further avenues of investigation.

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ISOLATION OF *RICKETTSIA TSUTSUGAMUSHI* FROM RODENTS FROM THAILAND

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Although scrub typhus is widely distributed in Burma and Malaya, there have been few definite records of the occurrence of the disease in neighboring Thailand. Audy (1949) cites Japanese accounts of wartime scrub typhus in Nipponese troops in the Kwe Noi Valley. Thaineua in 1953 reported the first serologically confirmed case of the disease in Thailand. The present paper reports the first isolation of *Rickettsia tsutsugamushi*, the etiological agent of scrub typhus, from tissues of Thai rodents (*Rattus* and *Bandicota*).

The rodents were collected during surveys on plague undertaken by members of the U. S. Special Technical and Economic Mission to Thailand in 1952.

MATERIALS AND METHODS

A preliminary chigger survey was undertaken in order to select sites likely to harbor rats infected with *R. tsutsugamushi*. Preserved samples of chiggers were sent to the U. S. Army Medical Research Unit and the Colonial Office Medical Research Unit at Kuala Lumpur, Malaya, and these organizations determined that a known vector, *Trombicula deliensis*, was prevalent in the Nakhon Pathom, Muang Lampaya area. Rats and ground squirrels from this locality were flown to Washington, D. C., where rickettsial isolation studies were undertaken at the Army Medical Service Graduate School.

The livers and spleens of the Thai rodents were pooled according to species and locality. Generally two or three rodents were used per pool of tissues, but later, tissues from a single rodent were employed in order to obtain data on the number of rodents infected. Other details concerning the pools are shown in Table 1. Ten per cent suspensions of these tissue pools were prepared in saline and inoculated intraperitoneally into laboratory white mice (Bagg strain). The inoculated mice were observed daily for external signs of disease. Animals were routinely sacrificed and passaged on the fourteenth day, unless frank illness necessitated earlier passage. If no evidence of infection was noted in the test mice the line was discontinued after the third passage. Impression smears were prepared from uncut surfaces of spleen and liver and from peritoneal scrapings of the passage mice. These preparations were stained by Giemsa's method and examined microscopically for the presence of cytoplasmic rickettsia-like bodies. Upon the isolation in mice of a disease-producing agent suggestive of *R. tsutsugamushi*, the following procedures were used: mice inoculated with lethal doses

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TABLE 1

Attempts at isolation of Rickettsia tsutsugamushi *from Thai rodents*

NUMBER OF ANIMALS TESTED	*Bandicota* sp.	*Rattus rattus thai*	*Menetes berdmorei*
Total	15	8	4
Pools of 2–3	4	2	2
Individual	3	4	0
Positive	1 pool of 3	1 pool of 2	0

of such agents were given daily doses of 2.5 mg. of chloramphenicol in their drinking water for a period of 17 days. Animals surviving with the aid of such chemoprophylaxis underwent challenge with the homologous strain 28 days after the original infection. In cross immunity tests, 25–250 mouse LD_{50} of yolk sac suspension of the Karp strain of *R. tsutsugamushi* were used for heterologous challenge of these mice on the 28th day following homologous challenge. Mice rendered immune to the well-studied Karp strain (Smadel *et al.*, 1949) by means of infection and chemoprophylaxis were challenged with 3100 LD_{50} of the *Bandicota* strain or with 4100 LD_{50} of the *Rattus* strain. Complement fixation tests were performed according to the technic employed in the Virus Department of the Army Medical Service Graduate School. Sera from guinea pigs which had survived following concurrent intraperitoneal and intracerebral inoculation with mouse tissues infected with the Thai strains were titrated in the presence of four units of scrub typhus soluble antigen (Topping and Sheppard, 1946) (Karp strain) prepared from yolk sacs.

RESULTS

Two strains of *Rickettsia tsutsugamushi* were isolated from the rodents listed in Table 1. One of these was obtained from *Rattus rattus thai* (No. B-10340) and the other from *Bandicota* species (No. B-10341), probably *B. bengalensis*. The *Rattus* strain was derived from pooled livers and spleens from two rats, while the *Bandicota* strain was obtained from a pool of these tissues from three rodents. Ten per cent suspensions of tissues from animals infected with the *Rattus* strain invariably killed laboratory mice between the ninth and sixteenth days after inoculation and showed a titration endpoint of about $10^{-4.6}$. The *Bandicota* strain, in early passage, killed the majority of mice inoculated with 10 per cent suspensions of infected tissues, but after four or five passages killed with regularity and had titration endpoints of $10^{-4.5}$. Both strains produced pathological changes in white mice typical of those of scrub typhus, including serofibrinous peritoneal exudate, enlarged spleens, subcutaneous injection and swollen inguinal lymph nodes. Intracellular rickettsiae were regularly demonstrated in Giemsa-stained smears.

Mice inoculated with 50–400 LD_{50} of the *Bandicota* or *Rattus* agent survived when treated orally with chloramphenicol. Mice which survived infection with the Thai agents were resistant to challenge with the homologous strain. Such survivors resisted challenge with 25–250 LD_{50} of the Karp strain of *R. tsutsuga-*

TABLE 2

Identification of Thai strains of Rickettsia tsutsugamushi

EXPERIMENT NUMBER	IMMUNIZING STRAIN	CHALLENGE STRAIN	DILUTION OF CHALLENGE INOCULATION						ENDPOINT
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
I	*Bandicota* agent immune mice	Karp		0/15*	0/22				
	Rattus agent immune mice			0/8	1/8				
	Control mice		6/6	6/6	6/6	6/6	0/6	0/6	$10^{-4.5}$
II	Karp immune mice	*Bandicota* agent	0/9	0/12	0/6				
	Control mice		6/6	6/6	5/6	6/6	1/6	0/6	$10^{-4.5}$
III	Karp immune mice	*Rattus* agent	0/11	0/14	0/6				
	Control mice		6/6	6/6	6/6	5/6	2/6	0/4	$10^{-4.6}$

* Fraction represents number of animals dying/number challenged.

mushi, as shown in Table 2. Moreover, this table also provides data indicating that mice immune to the Karp strain withstood challenge with at least 3000 LD_{50} of the Thai strains.

Convalescent sera obtained from guinea pigs following recovery from infection with either the *Rattus* or *Bandicota* agents fixed complement specifically in the presence of scrub typhus soluble antigen. In each instance a complement fixation titer of 1/32 was obtained. Control tests with these sera and normal yolk sac antigen gave negative results.

DISCUSSION

The genus *Bandicota* consists of large rats which are considered important hosts of the vectors of scrubs typhus, infestations having been reported by members of the United States of America Typhus Commission in North Burma (Mackie *et al.*, 1946), by the British Field Typhus Research Team in Manipur (Audy *et al.*, 1947), and by Indian Scientists in Bengal (Krishnan *et al.*, 1949). Recovery of *Rickettsia tsutsugamushi* from the tissues of *Bandicota bengalensis* in lower Burma has been reported by Kalra (1949) and this has been referred to by Harrison and Audy (1951). However, in the two instances Kalra mentions, the tissues had been pooled with material from *Rattus* and hence there is some doubt about the source of the isolate.

The recovery of *R. tsutsugamushi* from *Rattus rattus thai* is in accord with the findings of other investigators, since the majority of isolations have been from *Rattus*, especially subspecies of *Rattus rattus*, as noted by Harrison and Audy (1951).

SUMMARY

The isolation of *Rickettsia tsutsugamushi* from tissues of Thai rodents is reported. *Rattus rattus thai* is shown to harbor the organism and definite evidence is presented that *Bandicota* species are infected. Data are presented showing that the isolated strains meet the criteria for *Rickettsia tsutsugamushi*, as noted from pathological findings, microscopic studies of smears, cross immunity experiments and serology. These constitute the first recorded isolations of the etiologic agent of scrub typhus from Thailand.

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NATURAL OCCURRENCE OF TULAREMIA IN THE LONE STAR TICK, *AMBLYOMMA AMERICANUM* (Linn.), AND IN DOGS IN ARKANSAS

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Since the discovery of tularemia, or "rabbit fever," in 1910 by Dr. George W. McCoy (1911, 1912) in ground squirrels of Tulare County, California, the number of cases reported in the United States has increased rapidly and during the past 25 years has averaged more than 1,000 per year.

Francis (1937) reported that wild rabbits and hares are the source of more than 90 per cent of all human tularemia cases in the United States. However, in Arkansas, data compiled by Washburn and Tuohy (1949) over a ten-year period indicate that tularemia in that State is mainly a tick-borne infection. Their data show that the highest incidence of tularemia in Arkansas occurs during the months of April through September and is in keeping with the seasonal history of the most abundant ticks within the State, not with the rabbit hunting season. Further epidemiological investigations reported in the same paper revealed that the greatest percentage of human tularemia cases had a history of tick bites (56 per cent) rather than contact with infected rabbits (31 per cent) or other causes (13 per cent). According to United States Public Health Service data, 1946–1950 (National Office of Vital Statistics, 1947–1951), Arkansas, with 949 cases reported during the five-year period, had the highest incidence of tularemia of any state.

As a consequence of these studies by Washburn and Tuohy, a joint project between the Communicable Disease Center and the Arkansas State Board of Health was established to study this problem. The objectives of a survey conducted in 1951 were to determine (a) the species of ticks naturally infected with tularemia and the importance of each; and (b) the species of ticks occurring in Arkansas, and the distribution, hosts, seasonal activity, and abundance of each.

Distribution and abundance of tick species. Four genera and five species of ticks were collected from 13 species of animals from June through August during the 1951 survey (Table 1). No attempt was made to collect the species which occurred on animals other than those listed or which did not attach to drags[1] (Fig. 1). Of the total ticks collected, 92.8 per cent were found to be lone star ticks, *Amblyomma americanum* (Linn.). These were collected in 62 counties (Fig. 2). The American dog tick, *Dermacentor variabilis* (Say), comprised 7.1 per cent of the total number collected and was taken in 64 counties (Fig. 3), showing a wider distribution within the State than the former species. The average number of lone star ticks per dog was 3.4 and of American dog ticks, 3.7. The remainder of the ticks collected, in order of decreasing numbers, were

[1] A "drag" is made by trailing a flag-like flannel over the surface of vegetation for a distance of 100 paces (approximately 100 yards) (Fig. 1).

TABLE 1

Total number of animals examined, drags made, and ticks collected (all stages), June–August 1951

SOURCE OF COLLECTION	NUMBER SOURCES EXAMINED	LONE STAR TICKS		AMERICAN DOG TICKS		NO. OF TICKS OF OTHER SPECIES§
		Avg. no. per host or drag	% of total ticks from host or drag	Avg. no. per host or drag	% of total ticks from host or drag	
Drag	4,271	21.1	99.6	0.1	0.4	0
Dogs	1,899	3.4	47.8	3.7	52.0	17
Cattle	1,218	1.2	97.5	†	2.5	0
Horses and mules	286	5.4	96.4	0.2	3.6	0
Poultry	94	0.3	*	0	0	0
Rabbits	87	1.3	47.1	0	0	124
Hogs	41	1.8	66.4	0.9	33.6	0
Goats	40	0.7	96.3	†	3.7	0
Quail	10	3.7	*	0	*	0
Cats	7	*	*	*	*	0
Squirrels	4	*	*	*	*	0
Deer	3	*	*	*	*	0
Raccoons	2	*	*	*	*	0
Fox	1	*	*	*	*	0
Other sources‡	43	—	—	—	—	0

§ Species as follows: rabbit ticks: 122 from rabbits; black legged ticks: 15 from dogs, 2 from rabbits; Gulf Coast ticks: 2 from dogs.

† Less than 0.05.

* Insufficient numbers examined to give reliable percentage or average.

‡ Hosts or sources from which no ticks were taken are: 19 chicken houses, 12 meadow larks, 8 doves, 3 owls, 1 hawk.

the rabbit tick, *Haemaphysalis leporis-palustris* (Packard), the black legged or deer tick, *Ixodes scapularis* (Say), and the Gulf Coast tick, *Amblyomma maculatum* Koch. *A. maculatum* was collected in Washington county in northwest Arkansas, in Polk County in the midwestern part of the state, in Drew and Ashley Counties in the southeastern part and in St. Frances County in the mid-eastern section.[2] The low number of deer ticks collected was due to the fact that the extensive survey work was made during late spring, summer and early fall, when adults of this species were inactive. The relatively small number of examinations of rabbits, chief host of the rabbit tick, accounts for the small collections of this tick; this species does not attach readily to drags.

One lot of lone star ticks was found infected with *Pasteurella tularensis*, the causative organism of tularemia, in July 1951.[3] This infected lot was composed of 9 males, 8 females and 5 nymphs taken from 4 dogs in Marion County, Arkansas.

Tularemia infection in A. americanum. In view of the findings in 1951, the

[2] The *A. maculatum* were identified by Dr. Glen M. Kohls, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

[3] Tests were made by animal inoculation by personnel of the San Francisco Field Station under guidance of Mr. Frank Prince and Dr. Stewart Quan.

FIG. 1. Examining "drag" for ticks in typical lonestar tick habitat

investigations in Arkansas were continued another year. Investigations were resumed April 14, 1952, but not on a state-wide basis as was done previously. Areas in which the studies were to continue were selected for past high incidence of tularemia and for great density of the tick populations. Each area in which human cases of tularemia occurred in 1952 was subjected to intensive studies of suspected insect and mammal life occurring within the areas. All ticks collected in 1952 were packed in plastic collecting tubes and shipped alive by air mail.

Five lots of tularemia-infected lone star ticks were found (Table 2) by animal-inoculation test in a total collection of 12,845 specimens composing 242 lots of this species tested. Approximately 2,201 American dog ticks, 1,494 rabbit ticks, and 142 black legged ticks composed of all stages were also subjected to animal inoculation tests for tularemia but none of these specimens was found naturally infected by the procedure used.

The positive lone star ticks collected May 5, 1952 (Table 2) in Montgomery County were in a single pool collected in 12 drags, all of which were made within a six-mile area. Extensive collecting was done in an attempt to determine from which of these areas the positive ticks came. The points of the initial collections were thoroughly restudied at intervals, collections being made in every accessible tick habitat for a mile in radius around these central points. A total of 863 lone star ticks, 99 American dog ticks and 10 rabbit ticks were collected in 252 drags and from 10 dogs, 1 horse, 4 cows and 1 rabbit without success in relocating the focus.

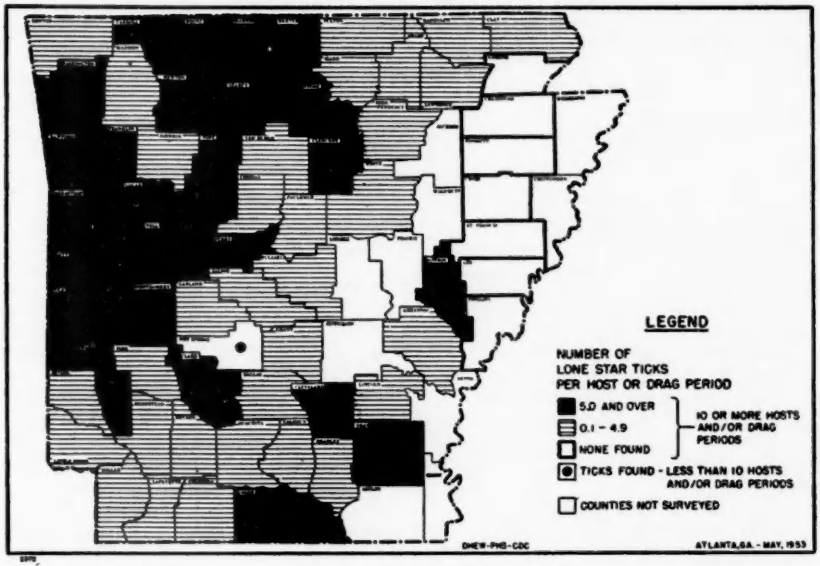

FIG. 2. Collection records of lonestar tick. In general, these indicate relatively large populations of this tick in the northwestern part of Arkansas and relative scarcity in the eastern part. The true status for any one county is probably closer to the average of that and surrounding counties than as shown on the map.

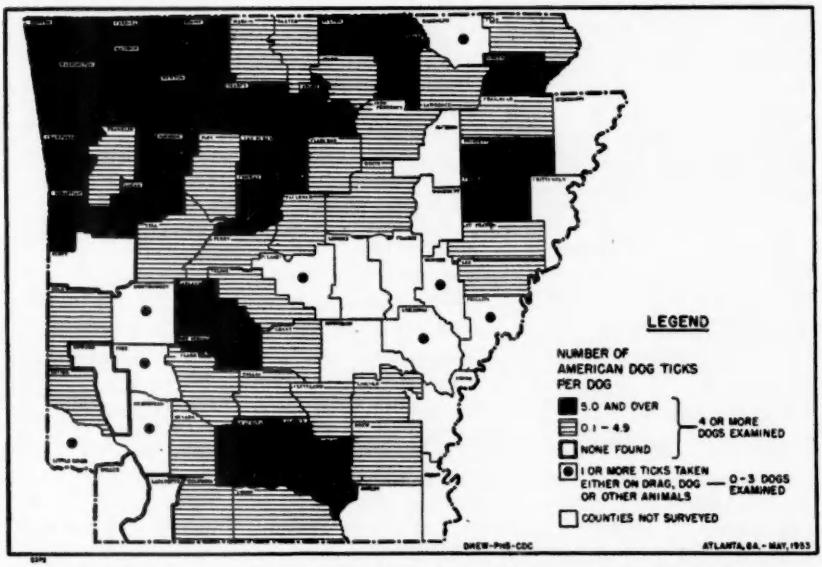

FIG. 3. Collection records of the American dog tick. In general it appears to be more abundant in the northernmost part of Arkansas than in the southern part. The true status for any one county is probably closer to the average of that and surrounding counties than as shown on the map.

TABLE 2

Naturally tularemia-infected lone star ticks collected, May–July 1952

DATE COLLECTED	SOURCE OF COLLECTION	SPECIMENS IN POSITIVE LOTS: STAGE AND NUMBER				NEGATIVE SPECIMENS COLLECTED FROM SAME HOST: STAGE AND NUMBER								COUNTY (ARKANSAS)
		Lone star ticks				Lone star ticks				American dog ticks				
		L	N	M	F	L	N	M	F	L	N	M	F	
May 5	12 drags	—	—	—	15	—	15	9	2	—	—	—	—	Montgomery
May 26	2 dogs	—	—	—	38	—	—	50	—	—	—	7	4	Garland
July 11	2 cows	—	1	36	27	—	—	—	—	—	—	—	1	
	10 drags	—	1	—	—	—	—	—	—	—	—	—	—	Washington
July 29	2 cows*	1	11	23	22	—	—	—	—	—	—	—	—	Benton
	5 dogs*	12	11	31	7	—	—	—	—	—	—	92	49	Benton
Total.....		13	24	90	109	—	15	59	2	—	—	99	54	

* Ticks collected from these 2 cows and 5 dogs were from the same farmstead.

The infected lone star ticks collected May 26 from 2 dogs in Garland County (Table 2) were possibly from a much wider geographical area than were the collections by drags in Montgomery County since dogs travel and hunt extensively.

Two hundred and ninety American dog ticks, 3,880 lone star ticks and 318 rabbit ticks were collected within a 2-mile radius of the positive area in Garland Co. but no positive specimens were obtained in this area after the original collection of May 26, 1952.

Tularemia in dogs and cattle

Blood specimens, taken from some individual hosts from which the positive tick collections were removed, were submitted for tests to determine by the agglutination procedure whether or not tularemia antibodies were present.[4]

Cow No. 3 (Table 3) was one of two from which the positive ticks were collected July 11 in Washington County. Upon returning to the herd to obtain a blood specimen, it was not possible to identify with certainty the second cow from which the positive ticks were removed. However, blood specimens from cow No. 3 and from three other cows from the same herd were submitted for tularemia and brucellosis tests. The specimen from cow No. 3 was found positive for tularemia at a dilution of 1:80. Of the other three submitted, two were found positive for tularemia in dilutions of 1:40, while the remaining cow was found to be negative for tularemia but positive for brucellosis in a dilution of

[4] Tests were made by personnel of the Hygienic Laboratory, Arkansas State Board of Health, Little Rock, Arkansas. To determine the presence or absence of tularemia antibodies, all sera were screened by means of a rapid slide agglutination test. If clumping was seen in either one of two slide test dilution mixtures (1:40; 1:160), a tube was set up using dilutions of 1:20 and 1:640, or higher if necessary. Tube agglutinations were incubated in a water bath at 52°C. overnight and read. Tularemia antigens used in these tests were prepared by the laboratory, using the National Institute of Health stock strain No. 38 *Pasteurella tularensis*.

TABLE 3

Host blood specimens submitted for tularemia agglutination tests from which positive ticks were collected

COUNTY	HOST	DATE TESTED (1952)	TULAREMIA AGGLUTINATION TITERS	AGE OF ANIMALS
Cow:				
Washington	No. 3	August 5	1:80	Adult
Benton	No. 4	August 15	1:80	Adult
	No. 5	August 15	1:80	Adult
Dog:				
Garland	No. 1	July 14	1:320	1½ yr.
		July 30	1:160	1½ yr.
		August 19	1:160	1½ yr.
	No. 2	July 14	1:320	2 yr.
		July 30	1:160	2 yr.
		August 19	1:320	2 yr.
Benton	No. 6	August 15	1:40	Adult
	No. 7	August 15	Neg.	Adult
	No. 8	August 15	1:320	Adult
	No. 9	August 15	1.640	Adult
	No. 10	August 15	1:160	Adult

1:40. Sera of six of the seven dogs tested were positive to the agglutination test for tularemia in titres ranging from 1:40 to 1:640.

CONCLUSIONS

Lone star ticks are a common vector of tularemia in Arkansas and similar ecological areas. Dogs may be an important source of infection to ticks and are certainly important as disseminators of ticks which may be infected. The exact role of cattle as hosts to the tularemia organisms is not clear.

SUMMARY

Epidemiological investigations of tularemia in Arkansas revealed that the highest incidence of human tularemia within the State occurred during the months of April through September, with approximately 56 per cent of the yearly reported cases having a history of tick bite. These epidemiological investigations prompted a cooperative project between the Communicable Disease Center and the Arkansas State Board of Health.

Data collected for two consecutive years indicate that the lone star tick is the predominant tick in Arkansas, followed by the American dog tick during the summer, as represented by collections from cattle and dogs and by dragging. The American dog tick was found to be the more widely distributed of the two species within the State. The lone star tick predominated in the more mountainous and heavily wooded areas.

Pasteurella tularensis was isolated from six different lots of lone star ticks (1

in 1951 and 5 in 1952) collected from dogs and cattle and by drags. Two of the positive lots were composed entirely of adult female lone star ticks. Though one large collection of American dog ticks was removed from the same hosts at the time the positive lone star ticks were collected, none of the American dog tick collections was found to be positive for tularemia.

Blood specimens were taken from the hosts from which the positive lots of ticks were removed. Of sera of seven dogs submitted for the tularemia agglutination tests, six were found positive in dilutions from 1:40 to 1:640. Three cow blood specimens gave positive tularemia reactions in dilutions of 1:80.

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THE OCCURRENCE AND MOVEMENT OF *PERIPLANETA AMERICANA* (L.) WITHIN AN URBAN SEWERAGE SYSTEM

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Although cockroaches have long been suspected as possible vectors of enteric infections in human beings, there is as yet no proof that these insects are concerned in the spread of salmonellosis or shigellosis. Laboratory studies of several investigators (Olson and Rueger, 1950; Jung and Shaffer, 1952) have shown that the American roach *Periplaneta americana*, the oriental roach *Blatta orientalis*, and the German roach *Blattella germanica* can harbor *Salmonella* for periods of 7 to 10, 20, and 12 days, respectively. Viable organisms of *Salmonella oranienburg* were recovered for 78 days from the pronotum of artificially contaminated *P. americana* by Olson and Rueger (1950). Other workers (Mackerras and Mackerras, 1949) have implicated roaches as secondary foci of infection in hospital outbreaks of gastro-enteritis among infants in Australia, while in San Antonio, Texas, Bitter and Williams (1949) reported *P. americana* taken from sewer manholes to be contaminated with *Salmonella schottmuelleri*, *Salmonella oranienburg*, and *Salmonella bredeney*. Syverton *et al.* (1952) recorded the isolation of four strains of poliomyelitis virus from *P. americana*, *Blattella germanica*, and *Supella supellectilium* captured on the premises of patients ill with paralytic poliomyelitis.

These instances coupled with the prevalence and movement of cockroaches in and around food-handling establishments, residences and waste disposal sites have focused further attention upon the importance of these insects as possible vectors of enteric infections. Concurrent with this interest is the renewed effort by communities to control roaches within city sewerage systems (Gary, 1950). The heavy roach infestations within such systems combined with the availability of human wastes are factors which conceivably could constitute a potential hazard to the health of a community.

Since it has been demonstrated that roaches resident within the sewerage systems can become contaminated with pathogenic organisms, the next step in the mode of spread of the pathogens would involve the degree of dispersion of the infected roaches and the contact between the insects and the human population. To obtain information on the dispersion of roaches within and from a sewerage system, a study was conducted at Phoenix, Arizona, in October 1952. Previous surveys of 22 selected manholes in that city for a seven-week period had shown a weekly average of 92 to 143 specimens per manhole with all roaches being *P. americana*.

METHODS

Approximately 6,500 American roaches, *P. americana*, were trapped in sewer manholes, tagged with P^{32}, and then released at selected sites. To capture the specimens, a quart jar fitted with a plastic screen cone and baited with overripe bananas was placed on its side in each of 9 manholes. A total of 18 traps was operated for 12 days to collect the desired number of specimens. Prior to marking, the roaches were maintained in 18-inch square screen cages on a diet of banana and powdered milk.

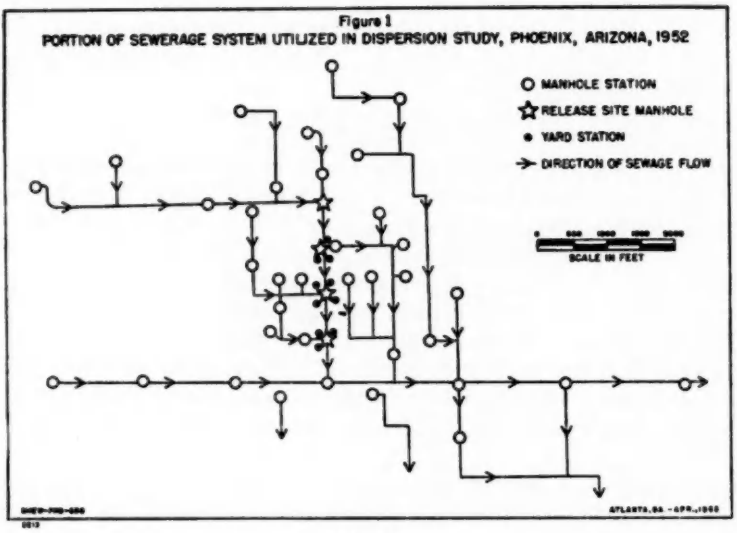

FIG. 1

To tag the roaches, a radioactive casein solution containing 10 microcuries of P^{32} per milliliter was sprayed upon the specimens under confinement.[1] The spray mixture contained equal parts of a 10 per cent casein solution and a P^{32} solution, the former being included to assure adhesion of the spray to the integument of the roach. The initial step in the treatment of the roaches was to place 1,000 to 1,500 specimens in a 10 or 20 gallon garbage container which was covered by a transparent plastic lid. The latter was equipped with an exhaust filter and a center hole for nozzle insertion. The spray was then introduced by means of a nasal syringe attached to a small air compressor. A total of 40 to 50 milliliters of spray solution sufficed for each can application, the operation requiring approximately five minutes.

Following the application of the spray, the container was allowed to remain undisturbed for fifteen minutes. At the end of that time, the plastic lid was

[1] Special thanks are due Dr. R. W. Fay and Mr. J. A. Jensen, Technical Development Laboratories, Savannah, Georgia, for developing this technique of tagging cockroaches with radioactive phosphorus.

TABLE 1

Number of Periplaneta americana *released and recaptured, Phoenix, Arizona, 1952*

	COLLECTION SITE		
	Release point	Manhole	Yard
Radioactive roaches released	6,500	—	—
Total number collections	17	408	120
Non-radioactive roaches collected	23	16,021	0
Radioactive roaches collected	906	0	1

replaced by the standard garbage can cover. When treatment of all roaches was completed, the contained specimens were transported to the liberation sites which consisted of four manholes one block apart and serving the same trunk line. Release of the specimens occurred at dusk, the container being lowered into the manhole and the lid removed. The opened container remained in the manhole for a 24-hour period.

For recovery of the tagged specimens, 34 traps were located in sewer manholes within a one-mile radius of the four release sites, the majority of the stations being within the 0.5 mile radius (Fig. 1). On the basis of the direction of sewage flow, stations were selected at manholes below and above the release points and at manholes located on secondary lines. In addition to the manhole sites, 10 traps were placed on premises in the blocks immediately adjacent to four liberation sites.

Collection of specimens was effected at each station for 8½ weeks following the release of the tagged roaches, a total of 12 samples being procured from each manhole. Radioactive roaches were detected by examining all samples with a laboratory or field count rate meter equipped with a thin-walled Geiger tube.

RESULTS

The collection data are summarized in Table 1. As is apparent, only one tagged specimen was recovered from sites other than the release point. Despite the absence of marked specimens, all manhole stations yielded *P. americana*, the average number per collection being 39 specimens. Only one specimen was trapped in the 10 yard stations but this roach was radioactive.

Three of the four release sites were trapped to provide a total of 929 roaches in 17 collections or an average of 54 specimens per sample. Of this number, 97.5 per cent were radioactive, thus demonstrating that the method of tagging had been effective. Further substantiation of this aspect was shown by the recovery of tagged roaches throughout the 8½ week period. Specimens captured 39 days after release displayed counts of 1,000 to 6,000 per minute.

DISCUSSION

The lack of movement exhibited by the marked *P. americana* was contrary to the degree of dispersion anticipated. Gould and Deay (1940) have reported migration of *P. americana* from restaurants and dumps and this species is

generally considered capable of nocturnal flight. Other unconfirmed lay reports have indicated roach movement from sewer manholes. While such information does not predict roach movement within a sewerage system, the favorable conditions present within sewers, together with the observed tendencies of the species to disperse, does lead to the assumption that an interconnecting network of sewer lines within a community could easily serve as a common channel for cross migration of roaches between the different parts of the city. The absence of any positive roaches in manholes only one block distant from the release point thus appears incongruous with the observed migratory tendencies of *P. americana*.

The conclusion derived from the experimental evidence is that *P. americana* does not disperse throughout the urban sewerage system of Phoenix, Arizona. Apparently conditions wholly satisfactory to the survival and propagation of the species exist at the manhole sites and their immediate vicinity. Actually, the creation of an induced population pressure by introduction of 1,000–1,500 roaches per release manhole was originally viewed with concern because it was feared this condition would force a greater degree of migration than would normally occur with a roach population.

If *P. americana* does not migrate between manholes, the question arises as to other avenues of dispersion. Two possibilities exist: 1) localized movement from manhole to lateral lines serving residences, and 2) escape from manholes and dispersion above ground. The present data give no clue as to the amount of dispersal in lateral sewer lines. However, since roaches occasionally enter residences via house drains, it is probable that limited movement of this type does occur. Moreover, based on the number of residences within the blocks surrounding the release manholes, there existed in the immediate environs considerable linear footage of lateral lines which, by furnishing extensive roach harborage, could contain heavy roach populations within a relatively confined area.

Evidence of roach dispersion above ground is indicated by the capture of one marked roach in a residential front yard 60 feet from the nearest release site. Possibly a greater amount of this movement occurred than the data reveal inasmuch as the 10 traps failed to capture a single unmarked specimen. This could well be an indication that the trapping technic was inadequate or that roach escape from the manholes and movement above ground were at a minimum.

Aside from the previous alternatives, there is the possibility that seasonal environmental factors might exert some effect upon roach movement. Only further studies during different seasons of the year can shed light on this aspect. Also of positive significance is the functional aspect of the sewerage system. At Phoenix, Arizona, the sewer mains handle sewage alone. While the arrangement is common in the United States, a number of cities possess systems which combine sewage and storm drainage. Under the latter condition, the intermittent flushing of the system could provide a means of conveying roaches from one area to another within the sewer mains.

The results reported tend to raise a question as to the relative importance

of roaches as a means of disseminating disease pathogens within the sewerage system and from such locations to human habitations. Further evidence discrediting the concept is the finding that the roach populations in sewer manholes are composed of one species, *P. americana*, whereas the predominant species taken in homes have been *Supella supellectilium* and *Blattella germanica*. This same condition of species distribution has been reported from Tyler, Texas (Anonymous, 1953).

In summary, the available evidence indicates *P. americana* to be the predominant roach in the sewerage system, the species apparently remaining at or near manhole sites without displaying tendencies to disperse throughout the system. Further experimentation appears desirable to substantiate and expand the previous findings, and to appraise the influence of seasonal environmental factors upon roach dispersion.

SUMMARY

A release of 6,500 radioactive American cockroaches, *Periplaneta americana*, in sewer manholes at Phoenix, Arizona, revealed no movement of marked specimens within the sewerage system. Marked roaches were recovered from three release sites for 8½ weeks. One tagged specimen was trapped at a yard site 60 feet from the release site. *P. americana* is indicated as the predominant roach in the sewerage system but not in homes.

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DUNG BEETLES (COLEOPTERA, SCARABAEIDAE) AND OTHER INSECTS IN RELATION TO HUMAN FECES IN A HOOKWORM AREA OF SOUTHERN GEORGIA

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The fate of human feces deposited on soil is an important consideration in epidemiological studies of hookworm and certain other helminthic infections in which soil-contamination with eggs or larvae excreted in feces is essential to dissemination. During the summer months in southeastern Georgia human stools are seldom in evidence in areas where such deposition occurs, and the sites are usually marked by disturbed soil that indicates burial of the feces by insects. Investigation revealed that dung beetles were responsible and led to the observations here reported.

The habits of scarabaeid dung beetles ("tumble-bugs," dung-burying beetles, and other coprophagous species) have long made them of interest to the naturalist and ecologist (Fabre, 1894, 1897, 1918; Lindquist, 1933, 1935; Cooper, 1938; Mohr, 1943). Their role as hosts of helminths (Hall, 1929) has brought them to the attention of parasitologists. Most biological studies have been concerned with their relation to animal dung, especially cattle droppings, which serves as food for both the adult and larval stages. Less attention has been given to their relation to human feces. Howard (1900) listed 11 species of Scarabaeidae among 44 species of Coleoptera found in surveys of the insect fauna of human excrement, Stiles and Hassall (1928) included a few more records, and Chandler (1929) recognized their importance in disposing of excrement in India.

The observations here presented were made principally in the vicinity of Waycross, Georgia, in July, 1951. They constitute a preliminary study concerning the general aspects of the relation of dung beetles and other insects to human stools.

Acknowledgement is made to Dr. Paul C. Beaver, a colleague who stimulated the writer's interest in the subject and contributed trap collections, and to Dr. John E. McCroan, Epidemiologist, and the Georgia Department of Public Health for supporting the project and providing facilities. Identifications of the insects collected were confirmed by Dr. O. L. Cartwright, Dr. H. R. Dodge, Mr. and Mrs. H. F. Howden, Dr. M. W. Sanderson, and Dr. W. C. Stehr, whose kind interest and assistance are also gratefully acknowledged.

METHODS

Direct observations in the field, feces-baited trap collections, and a few laboratory observations form the basis of this report.

Human stools, collected when passed in half-pint cardboard containers, were used fresh or after several days' storage at refrigerator temperatures. Specimens were placed on the ground or in traps in a variety of localities: (*A*) a fenced

rural graveyard surrounded by long-leaf pine forest two miles from town, with sandy soil; (*B*) an open field one mile from town, with low herbaceous vegetation and barren areas in sandy soil; (*C*) woods next to field *B*, with dense undergrowth and humus-laden sandy soil; (*D*) a pine-woods and pecan-orchard area near a small stream in the outskirts of town, with humus-laden sandy soil; and (*E*) the laboratory grounds in town, on a tree-shaded lawn with dusty-surfaced sandy soil.

Stool specimens placed on the ground were watched continuously for a period of several hours and direct observations recorded on the insects attracted. In some cases a series of timed photographs was made to record the rate of disappearance of the specimen. Other specimens were left in the field and the sites revisited at intervals. Excavation of the site provided additional data on the burial of stools and the beetles concerned. Most direct observations were made during daylight hours.

The form of trap found effective for coprophilous beetles was a gallon syrup can baited with human feces. The can was imbedded up to its rim in soil (to permit insects to enter readily by walking as well as by flying), with the bait supported at ground level on a central pedestal. A one-inch layer of soil in the bottom of the can served as a burrowing and hiding place for the trapped insects, which were readily recovered alive and free of excrement. Observations on the behavior of beetles entering this trap showed that it is efficient in retaining all except the smallest species, and yields of 50 to 100 or more specimens were commonly obtained in 3 to 15 hours. Other insects (ants, earwigs, etc., but not flies) were also found in the traps.

During the observation and collecting periods at Waycross (July 2–24) the weather was in general clear and warm, with occasional light afternoon rain. The following data (U. S. Weather Bureau and World Almanac) are of interest in relation to the activities of beetles: daily temperature maxima 89–102°F. (mean, 95.6), minima 62–75 (mean, 69.6); total precipitation 3.12 inches on 10 of the 23 days (daily mean 0.14, with one 1.4-inch shower). During the trapping period sunrise was at 5:30 a.m. (E.S.T.; approximate), sunset 7:30 p.m.; twilight began 3:30 a.m., ended 8:45 p.m.; moon ranged from full to last quarter.

Some observations were made in the laboratory on the behavior of beetles confined in one-gallon jars or cans containing 6 to 8 inches of soil, covered with cheesecloth, and provided with human feces.

FATE OF STOOLS DEPOSITED ON SOIL

Twenty specimens of human feces were placed on the soil in various situations at four of the localities listed above (9 at *A*, 8 at *B*, 1 at *C*, 2 at *E*), during a period of 8 days, July 2 to 9. With the exception of one set out at night, they were deposited between 10 a.m. and 2 p.m. Three were formed specimens, the others mushy or semiformed. They ranged in wet weight from 18 to 100 grams (the majority 40 to 80 gm.) and varied correspondingly in bulk (about 1 cc. per gram).

Five of these stools disappeared completely within 2.5 hours, five others com-

pletely or almost so within 7 hours (*i.e.*, by evening of the day they were put out), and two within 10 hours (by 11 p.m.). Three others persisted longer but were gone the next day. In two instances portions remained into the second day but were gone by the fourth day. Observations were discontinued on the remains of one specimen at location *A* after 3.5 hours, and one at site *E* when dry the morning after initial exposure (17 hours). One specimen placed at 10 p.m. at location *E* was undisturbed at midnight but gone the next morning.

The sites from which stools disappeared were usually marked by small mounds of loose soil in which a few small burrow-holes were often apparent (Figs. 1 and 2).

Of the twelve stools (5 at *A*, 7 at *B*) which persisted on the ground less than ten hours, nine had been placed in open sandy clearings, with at most low scattered vegetation and full exposure to sunlight. One had been placed in a grassy, partly shaded area near shrubs in the graveyard, another among tall weeds at a fence line of the field, and another in a tree-shaded area with tangled undergrowth near an open-back privy next to the graveyard.

The five stools that persisted longest were in or near dense vegetation (under a shrub at *A*, near the border of woods at *B*, in a thicket at *C*), under wooden steps of a building (*A*), or in a privy enclosure (*A*). Four of these were situated within 25 yards of, and one (*C*) about 200 yards from, sites where other stools disappeared rapidly.

Stools exposed to the sun began to show a dry outer crust and a darker color within the first hour; in cooler shaded areas these changes occurred more slowly (in 6 to 8 hours). Small remnants became quite dry overnight. Drying effects were, of course, delayed by rain. Little diminution in fecal mass was noted that could be attributed to the action of rain alone, but no heavy downpour occurred during the observation period. Chandler (1929) states that domestic animals, especially cattle, pigs and dogs, habitually devour human feces, but the stools exposed at Waycross were not disturbed in this way.

Continuous observation of six stools until their complete disappearance and excavation at the sites of 13 stools revealed that they were removed from the surface of the ground by dung beetles which rolled away balls of feces or buried feces at the site. Stools that were deposited where dung beetles were not abundant or active enough to bury them remained on the ground despite the feeding activities of flies, maggots and other insects.

The activity of insects around stools was dramatic in the promptness of arrival, the numbers attracted, and the rapidity with which the feces were removed. Within a few minutes after deposition, the scene sometimes became a mêlée of busy flies and beetles. Tumble-bugs quickly gathered up balls of feces and hurriedly rolled them away in all directions to bury them at a distance. Other dung beetles burrowed into or under the edges of the stool and worked from below, carrying portions of feces into the soil. When the beetles were numerous the stool was soon completely removed and nothing remained except the mound of soil thrown up and worked by the burying beetles. Movement of the soil was sometimes apparent as the beetles continued their subterranean

activities. One entire stool was completely gone in 75 minutes; another 40-gram specimen in 83 minutes; and a third, of 80 grams, in 143 minutes.

INSECTS ATTRACTED TO FECES

Coprophilic flies and beetles were the predominant insects attracted to exposed stools. Other arthropods, mostly nonspecific feeders on organic matter, were occasional, incidental, or local visitors. These included ants, earwigs, mole crickets, and cydnid bugs, caught overnight in feces-baited traps, and mites and springtails sometimes present at the sites of stools. A few predatory and parasitic wasps were also collected, and beetle-infesting mites were common. A total of 74 species of arthropods was found in association with human stools exposed on the ground or in beetle traps at Waycross. (All species collected will be listed in a separate publication.) Of these 46 were considered coprophagous and the others predatory or parasitic.

Nineteen species of flies were collected on stools without the use of fly-traps. During daylight hours filth flies (mainly *Sarcophaga*, *Phaenicia*, and *Callitroga*) were usually the first insects to arrive and start feeding on the moist feces. They walked on the stool sometimes for minutes at a time, and individuals often remained in the immediate vicinity and visited the feces repeatedly. Their visits varied in frequency and only a few incomplete counts were recorded (*e.g.*, an average of two flies a minute on a fresh partly-shaded stool in locality *C*; more than 75 flies in three hours on an unshaded stool in locality *A*). Commonly four to six would be on a small stool simultaneously. Flies would continue to visit the feces as long as any remained on the ground, but their numbers decreased as the feces dried and became crusted.

"Flesh flies" (sarcophagids, particularly the smaller species) were most constant in occurrence. As they fed they also deposited minute larvae which immediately burrowed into the stool. These were carried off in the feces by dung beetles, and partly grown larvae were sometimes found in the buried excrement. In stools remaining on the surface the larvae completed their development in a few days and crawled into the soil beneath the stool to pupate. (Five of the ten species captured were also reared from larvae in the stools.) Adults of a small borborid fly (*Borborus* sp.) were also regularly present on stools and sometimes were observed riding on the larger dung beetles or associated with them in the soil. No borborid larvae were detected.

Since it was soon apparent that beetles were responsible for the rapid removal of stools, they were collected and studied intensively to determine their identities, abundance, and relative significance. The 3126 specimens found in association with stools on the ground or in traps included 45 species representing five families. The percentage composition of the series of 2593 specimens recovered from traps was as follows: 89 dung beetles (19 species of Scarabaeidae), 8.8 hister beetles (8 species of Histeridae), 1.5 short-winged scavenger or rove beetles (12 species of Staphylinidae), 0.4 tiger beetles (2 species of Cicindelidae), 0.3 ground beetles (4 species of Carabidae). (The relative abundance of hister and rove beetles was probably slightly greater than indicated; no special effort was made to recover all minute specimens.)

Dung beetles, which outnumbered other beetles nearly 9 to 1, are the principal coprophagous members of this assemblage. Their habits and significance will be considered in detail. The hister and rove beetles (mostly small dark species, 1.5 to 6.5 mm. in length), although specifically attracted to excrement as well as to other decaying substances, are regarded as primarily predatory in habits (Fichter, 1949). No direct observations were made, but they presumably feed on maggots and other insects and hence are characteristic and significant members of coprophilic fauna. The incidental tiger beetles and ground beetles also presumably include predators and were perhaps attracted to the traps, like occasional spiders, because of the abundance of potential prey. While some of the beetles other than dung beetles may also eat feces, they are of no importance in the rapid removal of stools.

Dung beetles, rove beetles, and hister beetles were promptly attracted to fresh stools placed on the ground and sometimes began to arrive within a minute or two. They came by flight, usually upwind, and alighted on or near the stool, either directly or after passing and circling back. If they alighted within a few feet, they immediately walked to the stool. Arrival continued as long as excrement was present, and sometimes even after dung beetles had buried the stool, late comers would search and burrow into the soil. No attempt was made to determine how long beetles remained underground at the site; however, some dung-burying beetles were found in the soil as long as four days after initial exposure of the stool. Adult beetles fed on the feces, but no beetle eggs or larvae were detected in association with feces excavated at the site within four days. No further effort was made to find special breeding burrows in which the feces might be used, like animal dung, as provisions for the larval stages.

HABITS AND POTENTIALITIES OF DUNG BEETLES

The 19 species of dung beetles collected are divisible into three groups differing in habits which may effect the dispersal of microörganisms present in human feces: tumble-bugs, which remove feces in the form of balls that are buried away from the original site of deposition; "burying beetles," which bury feces directly at the site; and other species, which consume only small amounts of feces at the site without burying it. These groups were represented at Waycross by 5 species of tumble-bugs, 9 species of burying beetles, and 5 other species (listed in Table 1 with the exception of two burying beetles, *Geotrupes egeriei* Germ. and *Onthophagus tuberculifrons* Har., which did not appear in the tabulated collections but were represented by one and two specimens respectively, in other collections).

Species removing feces from the site

Tumble-bugs constituted 66 per cent of the dung beetles and 59 per cent of all beetles trapped. The five species found (Figs. 3–7) range from about 4 to 18.5 mm. in length and are uniformly black in color, except *Canthon laevis*, a large species which varies from green (subspecies *viridescens* Horn) to black. Differences in the relative abundance and time of activity of the species were observed and are indicated in the analysis of trap collections given below (*cf.* Table 1).

Upon reaching the stool, tumble-bugs sometimes pause to eat at the surface, burrow in, or immediately begin to gather up material to form a ball. A portion of excrement is separated from the mass by the use of the mandibles, the flattened shovel-like head, and the legs which pass the material backward under the insect to build up the ball. This is then rolled away over the surface of the ground, the beetle walking backward with its fore legs on the ground and pushing the ball with its middle and hind legs. As the ball rolls it becomes covered with a layer of sand or dirt. The ball is rolled a variable distance until a spot is selected for its burial. Burial occurred in either bare or grassy places, from 4 to 20 feet (and undoubtedly farther) from the original site (Figs. 16–21). One *C. laevis* was observed burrowing under the ball six feet from the original site, three minutes after removing it from the stool. (Cooper, 1938, mentions a similar observation, with burial of the ball in seven additional minutes; Lindquist, 1935, says that rolling may continue an hour or more.) No investigation was made concerning the final disposition of these balls, but it is known that the beetles bury balls of cow dung at depths of one to five inches for use as food for the adult or the larva, a single egg being deposited on one side of the ball after it is buried (Lindquist, 1935). The apparent advantage of removing feces from the site of deposition is to save it from the ravages of other beetles. Competition among the tumble-bugs themselves is sometimes keen, and one may purloin the ball of another while the owner is occupied underground preparing a burrow for its reception.

The balls formed by tumble-bugs ranged from 6 to 25 mm. in diameter and from 0.2 to 9 gm. in weight, varying with the size of the species and with individual circumstances. In one instance 8 large and 16 small balls were removed from a 50-gram stool, the first rolled away five minutes after deposition and the last 2 hours and 35 minutes later (an average of one every 6.5 minutes). In another experiment, balls removed from an 80-gram stool in 143 minutes weighed 34 grams collectively; this represents a rate of removal of about 0.25 gm. a minute.

The relative significance of tumble-bugs in removing feces obviously depends on their abundance and size (species). In the Waycross area they were very abundant and played a major role in this regard.

Species burying feces at the site

The scarabaeid beetles that bury feces at the site of stool-deposition comprised 31 per cent of the dung beetles and 28 per cent of all beetles found in feces-baited traps. The nine species encountered (Figs. 8–15) range in length from 2 to 30 mm. Most are black in color, but some are conspicously green (*Phanaeus* spp.) or greenish-black (*Geotrupes egeriei*). They differed in abundance, and some species came to stools promptly during the day, others only at night (*cf.* Table 1).

After arrival by flight, the burying beetles immediately burrow into or beneath the stool and, after preliminary feeding, proceed to excavate burrows and carry down feces from below. Their activity is responsible for the loose soil that soon becomes apparent at the edge of the stool and eventually alone remains to mark

the site as a low mound up to 25 cm. in diameter (Fig. 2). The beetles were found in the soil down to 23 cm. and within a radius of 20 cm. The course of their tunnels was sometimes evident. Usually a few openings were present at the surface, but the burrow-entrances are often occluded in loose soil. The diameters of the burrows range from a few millimeters to about 3 cm., depending upon the species responsible.

The amount of feces found buried by individual beetles varied with the size and habits of the species (*cf.* Table 2), from mere flecks of 0.1 gm. or less (*e.g.*, by *Ateuchus*) to masses 2.5 cm. in diameter and 6.5 cm. long and weighing 26 gm. (by *Pinotus*). Often no feces were detected in association with beetles excavated at the site, but laboratory experiments gave some indication of their individual capacity for the removal of human stools (*e.g.*, 15 gm. in 3 days by one *Phanaeus igneus*). Moreover, it is known that some species bury much larger amounts of animal manure—up to 30 gm. (dry weight) of cattle dung by *Phanaeus* and 130 gm. by *Pinotus* (Lindquist, 1933). Fabre (1897, 1918) credits *Geotrupes* with exceptional capacities for burying excrement and with particular importance because of a predilection for human feces as adult food. He cites an instance in which twelve of these beetles buried mule dung overnight at a rate of 60 cubic inches each. They leave most of the buried material unconsumed, emerging the next evening to seek fresh manure. While only one specimen of *Geotrupes* was found at Waycross, with no associated feces, this genus may be abundant at other times and places and play a major role in burying large amounts of feces.

Human feces carried down by burying beetles were recovered from the soil at depths of 5 to 23 cm. under the site of stools. (Lindquist records cattle dung buried by some species as deep as 53 cm.) No direct observations were made on the ultimate fate of the buried stools, but the beetles utilize either all or only a part of buried animal dung as food for the adults and as provisions for their larvae. The adult beetles were observed to eat human feces avidly and in appreciable amounts, sometimes continuing for hours at a time and passing excrement *per anum* which accumulated in long, unbroken strands.

The dung-burying beetles compete with and supplement the tumble-bugs in the removal of stools, especially overnight. They are the important agents that bury feces at the site of deposition.

Other coprophagous species

Although the small aphodiine beetles which feed and breed directly in cattle droppings may be very abundant and important members of the fauna in such excrement, they were encountered relatively infrequently at the stools or in the traps baited with human feces (*cf.* Table 1). Together with three specimens of trogid beetles, they constituted only about 3 per cent of the dung beetles and 2 per cent of all beetles in the traps. They were trapped mostly overnight (95 per cent).

Since these beetles are not known to bury excrement they were considered of no significance in the removal of stools at this place and time.

Feces-removing potentialities

The quantitative data obtained by trapping enable a crude estimate to be made of the potential amounts of feces that the dung-beetle population might remove in the collection area.

A series of trap-collections was made in an open field (locality *B*). On July 17 one trap exposed from 9 a.m. to 12 noon yielded a total of 139 scarabeid beetles. From July 19 to 24, three traps, placed within a radius of 50 feet, were exposed continuously and simultaneously. These yielded over 2,000 beetles during the six-day period, of which about 1,800 were dung beetles. The trapped specimens were collected and pooled between 5 and 7 p.m. and between 8 and 10 a.m., the daytime collecting periods ranging from 8 to 11 hours and overnight periods from 14 to 17 hours. The total trapping period, beginning July 17, thus included 54.5 hours in daytime and 75 hours in evening, night, and early morning: a total of 129.5 hours, of which 126.5 were consecutive over a period of six days. The human stools (about 50 to 80 grams each) used as bait were renewed when the catch was collected, except on two days when the same bait was used for both daytime and overnight collections. A total of 28 stool specimens were used for 19 daytime and 15 overnight collections. A summary of the dung beetles collected in this series of traps is presented in Table 1.

Table 1 (Columns *a* and *b*) shows that the total of 1973 scarabaeid beetles trapped, representing 17 species, included 1464 tumble-bugs (74.2 per cent; 5 species), 488 burying beetles (24.7 per cent; 7 species), and 21 other coprophags (1.1 per cent; 5 species). More beetles were caught during the day (62.2 per cent) than overnight (Columns *c* and *e*). Nine species were trapped in daytime, with tumble-bugs greatly predominating (*Canthon laevis* 64 per cent, *C. depressipennis* 23.5 per cent). Eight additional species were present in the total of 17 collected overnight, with tumble-bugs (47 per cent) and burying beetles nearly equal in number and with four species predominating (*C. probus*, 33 per cent; *Phanaeus igneus*, 19; *Onthophagus pennsylvanicus*, 14; *Ateuchus* (*Choeridium*) *lecontei*, 12). Variation in periodic collections is apparent (Columns *d* and *f*); only the dominant species were present every day or every night. Since periods of daylight and darkness were included in the "overnight" collecting periods, the records do not differentiate between nocturnal and crepuscular or early morning flight activity, but they do indicate specific differences. The feces-burying activity of the tumble-bugs corresponds to their flight activity, but the burying beetles may continue underground activity during the day after finding the stools at night. The largest collections of all dung beetles (from three traps) consisted of 270 specimens in daytime and 198 overnight (not consecutive). Column *g* shows the average number of each species caught in 24 hours (24/129.5 of total specimens). For all the scarabaeid species listed this average was 365.4.

These collection records were used to calculate the amount of feces which the beetles could remove from the surface of the ground. The data for each species of the tumble-bug and burying-beetle groups are summarized in Table 2, based on the average numbers caught in 24 hours and the estimated amount of feces

TABLE 1

*Dung beetles collected in feces-baited traps in open field, Waycross, Ga., July 17–24, 1951**

SPECIES (*a*)	TOTAL NUMBER (*b*)	DAYTIME†		OVERNIGHT‡		24-HOUR AVERAGE (*g*)
		Total (*c*)	Range§ (*d*)	Total (*e*)	Range§ (*f*)	
Canthon laevis (Drury)	819	788	66–160	31	0–11	151.7
C. depressipennis Lec.	352	288	9–100	64	4–36	65.2
C. probus (Germ.)	277	28	0–12	249	14–66	51.3
C. granulifer Schmidt	14	9	0–5	5	0–5	2.6
C. vigilans Lec.	2	0		2	0–1	0.4
Tumble-bugs, 5 spp.	1464	1113	76–236	351	19–108	271.1
Onthophagus pennsylvanicus Har.	167	65	0–22	102	3–38	30.9
O. hecate Panz.	30	21	0–11	9	0–6	5.6
Phanaeus igneus MacL.	152	13	0–4	139	11–45	28.1
Ph. vindex MacL.	14	13	0–5	1	0–1	2.6
Ateuchus lecontei (Har.)	90	0		90	3–28	16.7
Copris minutus (Drury)	30	0		30	0–23	5.6
Pinotus carolinus (Linn.)	5	0		5	0–2	0.9
Burying beetles, 7 spp.	488	112	5–33	376	32–117	90.4
Aphodius lividus (Oliv.)	9	3	0–2	6	0–4	1.7
A. campestris Blatch.	7	0		7	0–3	1.3
Ataenius platensis Blanch.	1	0		1	0–1	0.2
Ataenius cylindrus Horn	1	0		1	0–1	0.2
Troz asper Lec.	3	0		3	0–2	0.6
Other coprophags, 5 spp.	21	3	0–2	18	0–7	3.9
Total Scarabaeidae, 17 spp.	1973	1228	85–270	745	78–198	365.4

* Seven days: 1 trap July 17, 9 a.m. to 12 noon; 3 traps exposed continuously and simultaneously within 50-foot radius July 19, 10 a.m., to July 24, 4:30 p.m.

† Periods ranged from 3 to 11 hours between 8 a.m. and 7 p.m.; total, 5.45 hrs.

‡ Periods ranged from 14 to 17 hours between 5 p.m. and 10 a.m.; total, 75 hrs.

§ Minimum and maximum numbers in pooled catch during one day or night period.

individual beetles might bury (Columns *b* and *c*). The individual amounts are conservative estimates derived from field and laboratory observations, including in most cases the weighing of a few samples of feces removed by or found with the various species. No calculations are given for species which do not bury feces, nor for the small burying beetle *Onthophagus pennsylvanicus* in association with which no buried feces were detected. They are considered as consuming only relatively small amounts of feces which would not materially affect the crude totals presented here. The individual amounts given for other species may be generally regarded as minimal; much larger average amounts of cattle manure were found in the burrows of several species by Lindquist. The quantity of feces available for the beetles in competition with others undoubtedly influences the

TABLE 2

*Amounts of feces removable by dung beetles collected in traps in 24 hours**

BEETLES (NUMBER)		FECES (GRAMS, WET WEIGHT; APPROXIMATE)					
Species (*a*)	24-hour average (*b*)	Avg.† per beetle (*c*)	24-hr. Avg. (*d*)	Daytime		Overnight	
				Avg.‡ (*e*)	Range (*f*)	Avg.‡ (*g*)	Range (*h*)
Canthon laevis	151.7	3.0	455	438	198–480	17	0–33
C. depressipennis	65.2	0.5	33	27	5–50	6	2–18
C. probus	51.3	0.2	10	1	0–2	9	3–13
C. granulifer	2.6	(0.5)	1.3	0.8	0–3	0.5	0–3
C. vigilans	0.4	7.0	3	0		3	0–7
Tumble-bugs, 5 spp.	271.1		502	467	203–535	35	5–74
Onthophagus pennsylvanicus	30.9	?	Little				
O. hecate	5.6	(0.1)	0.6	0.4	0–1	0.2	0–0.6
Phanaeus igneus	28.1	7.0	197	17	0–28	180	77–315
Ph. vindex	2.6	7.0	18	17	0–35	1	0–7
Ateuchus lecontei	16.7	(0.1)	2	0		2	0.3–3
Copris minutus	5.6	1.0	6	0		6	0–23
Pinotus carolinus	0.9	24.0	22	0		22	0–48
Burying beetles, 7 spp.	90.4		246	35	0–64	211	77–396
Total, 12 spp.	361.5		748	502	203–599	246	82–470

* Based on data in Table 1.

† Estimates based on few weighings or, in parentheses, size.

‡ Calculated on basis of daytime/overnight ratio of beetles.

amount removed. The maximum for the burying beetles was probably not attained under the conditions of rapid removal prevailing in the observed instances.

The amounts of feces listed in Table 2 (amount per individual multiplied by number of beetles) are approximations to the nearest whole number. These calculations indicate (Column *d*) that the 361 beetles (average) caught in the traps in 24 hours would be capable of removing about 748 grams of human feces. About two-thirds (502 gm.) of this would be rolled away by tumble-bugs and buried at varying distances; the other third (246 gm.) would be buried directly at the site of deposition. If the relative abundance and activity of the various species during day and night hours be considered (Columns *e* and *g*), it is apparent that twice as much feces would be removed during daylight hours (502 gm.) as overnight (246 gm.), because of the relatively large numbers of *C. laevis* and despite the greater amount of feces buried individually by the nocturnal but comparatively infrequent *Pinotus carolinus*. *Phanaeus igneus* would account for 180 gm. of the feces buried overnight. The ranges of potential amounts of feces removed (Columns *f* and *h*, based on the numbers of beetles found in periodic collections) indicate the wide variation that could be expected from day

to day, from 82 gm. overnight to 599 gm. during the day, or 285 to 1069 gm. in 24 hours if the diurnal and nocturnal minima and maxima, respectively, occurred in succession.

In general, it can be seen that the beetle population present would result in most feces being removed from the site by tumble-bugs during the day and buried at the site by the burying beetles overnight, with the two common species *Canthon laevis* and *Phanaeus igneus* playing dominant and complementary roles in this regard and together accounting for 87.4 per cent of the quantity removed. The other 15 species collected would remove only comparatively small amounts of the total feces if enough were available for all.

Two successive trap collections were made also on a clearing near a small stream among pine and pecan trees (locality *D*) July 12 to 16, over a period of 76 hours, day and night. They yielded a total of 349 dung beetles. The same species of tumble-bugs and burying beetles (except *Copris*) were present as at locality *B*, but tumble-bugs constituted only 21 per cent of the total catch. Calculations like those in Tables 1 and 2 give a 24-hour average of 110 beetles (23 tumble-bugs, 75 burying beetles, 12 *Aphodius*), with a potential feces-removing capacity of about 168 grams (21 by tumble-bugs, 147 by burying beetles). The markedly lower figures for burying potentials reflect the smaller number of beetles and particularly the relatively few tumble-bugs (23:271, as compared with locality *B*) and only half as many *P. igneus* (14:28), per 24 hours. This illustrates the effect of local conditions in determining the beetle population available for feces disposal, although no observations were made in locality *D* on the fate of exposed stools. Individual stools could still be expected to disappear quickly under these conditions.

Since the species of dung beetles present varied in their feces-burying potentialities, the fate of stools differed according to the number and kinds of beetles which found them. In localities *A* and *B* stools in open situations were invariably visited by an abundance of capable species and were rapidly buried. Neighboring stools in sheltered or otherwise unfavorable places attracted fewer beetles and remained on the ground a day or more. On the laboratory grounds in town (locality *E*), a stool placed out at night attracted only the small and relatively inactive *Ateuchus lecontei* and remained virtually intact and dry the next day.

DISCUSSION

The observations in Georgia show that dung beetles can be important agents in the rapid removal and burial of human stools deposited on the ground. The amount of feces removed and the rate of removal from a given situation depend upon the number and species of beetles attracted and the sequence and rapidity of their arrival. These in turn will depend upon season, weather, time of day, presence of other food supply (especially cattler doppings) in the area, type of soil, and the character and accessibility of the place where feces are deposited. The effects of variation in these factors are evident in the detailed data presented for Waycross and in a few exploratory observations in other localities.

The trapping area at Waycross locality *B* was an open, uncultivated sandy field about 200 yards from woods. There were apparently no cattle within a half mile or more, but small amounts of swine and dog droppings and some human stools may have been available in the general area. The abundance of beetles indicates an ample food supply, probably animal manures, in the region as a whole. The composition and activity of the beetle population were undoubtedly conditioned by the season (July) and locality (southern Georgia). The results of trapping and the speculations based on them in regard to feces-removing potentialities are consistent with the field observations in the same area and in area *A* where exposed stools immediately attracted large numbers of beetles and were rapidly removed. Of the dung beetles present, three species—*Canthon laevis*, *C. depressipennis*, and *Phanaeus igneus* (together comprising 61 per cent of the dung beetles trapped)—were considered of outstanding importance because of their abundance, size, and capabilities. Seven additional species were abundant and active enough to be of appreciable significance, while the other nine were not sufficiently numerous or not capable of consuming enough feces to be of much significance.

Since a normal human stool rarely exceeds 150 grams, the beetles attracted to three traps on about 0.2 acre during 24 hours, capable of burying at least 748 grams of feces, could bury the daily excrement of a family of four to six persons. It is not surprising, therefore, that during summer in this section of Georgia human stools are rarely seen on the ground, and then only when freshly deposited (Beaver, 1952). Defecation on the ground is a common and regular practice among rural families. A defecation site in a yard of a family of seven in Atkinson County contained only a small portion of formed stool, and digging at the sites where the soil had been worked by beetles revealed a number of the dung-burying species.

In New Orleans, in a locality where only a few specimens of small dung beetles were attracted by five stools in July and October, the feces remained on the ground five to eight days.

Trap collections in a farmyard at Glenwood, Indiana, in late July yielded 936 dung beetles representing seven species, but 98 per cent of these were *Onthophagus* spp. and only three *Canthon laevis* were included. It is doubtful that exposed human stools would disappear rapidly in the trapping locality, especially with the competition provided by swine and cattle manure.

Records from Kansas and Texas (Lindquist 1933, 1935) show that dung beetles—including the large species with great feces-burying potentialities—may be very abundant in favorable localities. He found 120 to 2000 burrows per acre in pastures at various times of the year (*Canthon*, *Pinotus*, *Phanaeus*, and *Copris*). Lindquist's biological studies on five Texas species indicate that they are not very prolific (*e.g.*, a recorded maximum of 67 eggs by *Onthophagus anthracinus* in 42 days, 17 by *Phanaeus triangularis* in 135 days) and that the developmental period from egg to adult ranges from about three weeks to a year (3 to 10 weeks in four species). Some may produce three or four broods a year, others only one, but some adults may live several years. Some species are known

to hibernate in special burrows in more temperate regions, active periods occurring between April and October. In southern Texas frequent rains and high humidity increase their activity.

Comparison of the results of exposing stools at Waycross and in New Orleans indicates that dung beetles were the only arthropods responsible for the rapid removal and burial of human feces. Flies and ants were attracted in both areas, but stools were buried only at Waycross where dung beetles were abundant and not at New Orleans where no large beetles were active. Although Argentine ants were abundant on stools in New Orleans, they evidently carried away little, if any, feces. Maggots and pill-bugs were also numerous but consumed stools so slowly that dry feces remained a week or more. Other coprophagous insects were too few to be significant in either locality. While approximately a hundred species of arthropods were collected in association with stools in Georgia, Louisiana and Indiana, only half of these are considered coprophagous and the others predaceous or parasitic. Of those that eat feces, only tumble-bugs and some other dung beetles have habits which make them significant in the rapid burial of stools. Fabre long ago extolled their virtues as Nature's sanitarians. Where dung beetles are few or absent, where only the smaller species with limited capacities are present, or perhaps where a superabundance of animal droppings diverts and competes, stools can be expected to remain where deposited until gradually consumed by maggots and other arthropods over a week or longer and until the dry remmants are mixed with the surface soil.

Beetles rival flies in the numbers of species and individuals associated with human excrement. Howard (1900) recorded 77 species of Diptera in this category, including 36 reared from stools. The 60 species of Coleoptera found in the course of the present investigation and 31 others listed by Howard and Stiles and Hassall (1928) constitute a total of 91 reported from human feces in the United States. They include 33 dung beetles in the eastern and southern States. Undoubtedly other similar species are attracted to this source of food elsewhere in this country and the world over.

The role of coprophilic arthropods in the epidemiology of hookworm infection has been accorded some attention but requires further elucidation. Cort and Payne (1922a, b) noted that in sugar and cacao plantations in Trinidad, the rapid disappearance of human stools within 24 hours due to "heavy rains and the activity of the insects" was a factor in keeping defecation areas acceptable for repeated visits. This resulted in concentrated pollution of certain spots which thus became important sources of infection. Chandler (1929) observed in India that all traces of a stool were usually obliterated in a few hours due to the activities of dung beetles, and that these and other insects (especially ants and maggots) mixed the feces with, and aerated, the soil, thus improving the cultural conditions for hookworm larvae. On the other hand the same author observed that roaches devoured stools rapidly in coal mines, destroyed most of the hookworm eggs ingested and consequently acted as important agents of control. The relation of insects to stools may therefore affect in various ways the dissemination of hookworms and other enteric parasites. Whether the activities of dung beetles

assist or hinder the success of infective stages undoubtedly depends upon a combination of factors which differ according to the biology of the parasites. Burial of infected feces alone need not be detrimental to hookworm, since the larvae can hatch, develop, and migrate upward through as much as 36 inches of sandy soil (Payne 1923). However, burial and ingestion of feces by beetles in underground chambers may differ sufficiently from artificial burial to interfere with successful development of the worms. Burial of ova, like those of *Ascaris* and *Trichocephalus*, that require direct ingestion by the vertebrate host would remove them from potent situations. Full evaluation of the epidemiological significance of dung beetles will require studies concerning the direct effects on parasites ingested or buried in the soil and the indirect effects on the degree of exposure of man to infective stages.

CONCLUSIONS

The limited observations reported here have confirmed or established certain facts and suggest the following appraisal regarding the relation of dung beetles to human feces and public health:

1. Dung beetles are readily attracted by human feces as well as by animal manure.

2. The species attracted utilize human feces as food, at least as adults.

3. Certain species remove human feces from the surface of the ground and bury it either directly at, or varying distances from, the site of deposition (up to twenty feet away and probably farther). It is stored for food at varying depths down to an observed maximum of nine inches; burial as deep as 12 inches, and sometimes perhaps several feet, can be expected on the basis of the known habits of some species.

4. Species of dung beetles differ greatly in habit and in the amount of feces utilized. Some are diurnal foragers, others are nocturnal.

5. The species attracted may occur in sufficiently large numbers to remove and bury an entire stool within a few hours after deposition.

6. The amount of human feces that can be removed by dung beetles and the rate of removal depend on the number and kinds of beetles present, which vary with geographical region, season, and local circumstances. In rural and semi-rural areas during warm weather the beetles may be very abundant and capable of removing the daily excrement of an entire family from outdoor defecation areas.

7. The larger species of dung beetles excrete considerable amounts of feces during their feeding and burrowing activities. This, as well as the transport of mammalian feces to a distance, could conceivably be a factor in the dissemination of microörganisms present in human stools.

8. The survival of the eggs and larvae of enteric parasitic worms, such as *Ascaris*, hookworm, and *Strongyloides*, in buried feces and in the beetles may be of epidemiologic importance.

9. The rapid removal of stools by dung beetles may have indirect influence on the degree of pollution and worm-infestation of soil by maintaining defecation

sites in acceptable condition for repeated use and thus enhancing the infection-potential of particular spots.

SUMMARY

Observations on human stools exposed in the field at Waycross, Georgia, in July, revealed that they may be buried by dung beetles within a few hours. "Tumble-bugs" (5 species), which form balls of feces and roll them away for burial at a distance, were most abundant and important as agents of removal, particularly during the day. Nine other species, which bury feces directly and produce mounds of disturbed soil at the site of stool deposition, were also significant, especially at night. Five additional coprophagous scarabaeids, of slight importance, were among a total of 74 collected species of insects attracted to human feces. The rest included 26 predatory Coleoptera, 19 Diptera, a few other coprophagous species (ants, cydnid bugs, earwigs, mole crickets), and a few parasitic wasps. The beetles collected in Georgia, Louisiana and Indiana augment the total reported from human feces in the United States to at least 91 Coleoptera, including 33 Scarabaeidae. Collections in feces-baited traps in Georgia also indicated that in seven days sufficient dung beetles were attracted every 24 hours in an area of about 0.2 acre to dispose of about 750 grams of feces, with two species of greatest potential importance. Differences in capacities and abundance of various species, influenced by region, season and local circumstances, would determine the amount and rate of feces-disposal at any particular place. Since feces are buried at varying depths (observed maximum, 9 inches) and carried away from the original site (20 feet or more), dung beetles may constitute an important factor in the epidemiology of hookworm and other enteric parasites of man.

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PLATE I

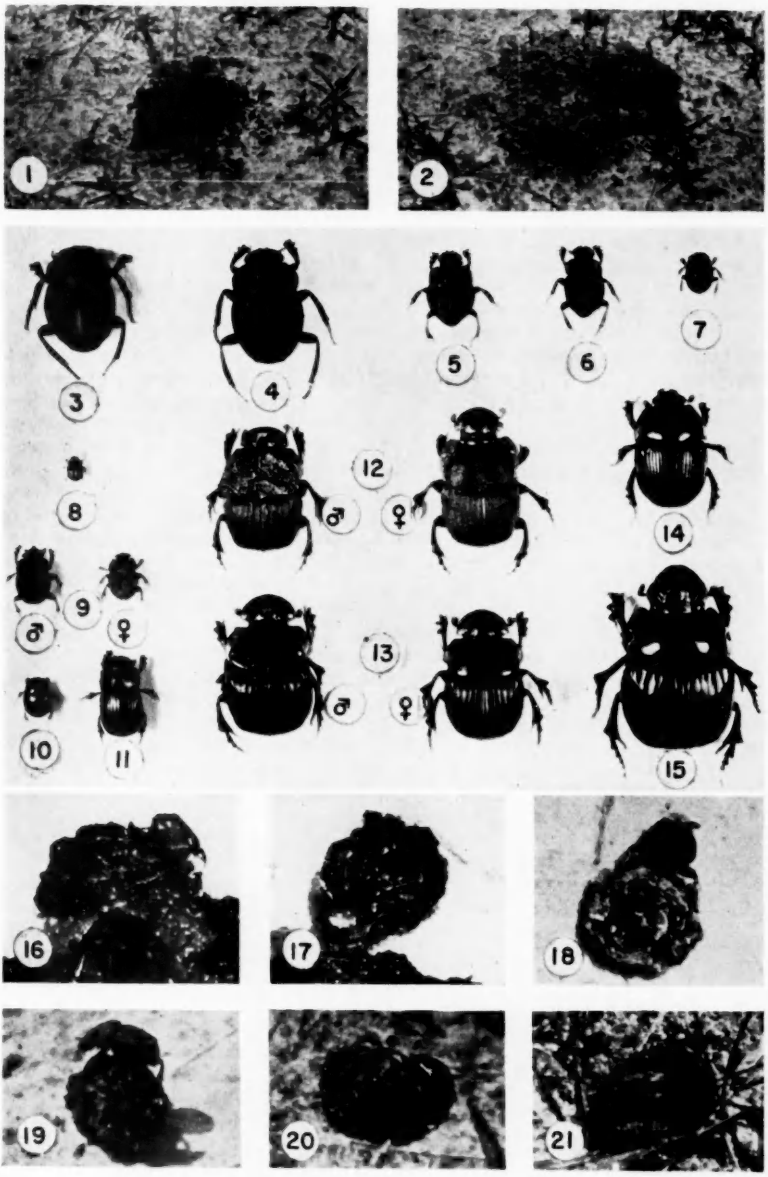
FIG. 1. Stool 10 minutes after deposition; a portion has already been removed by dung beetles.

FIG. 2. Site of same stool 75 minutes later; feces completely gone, mound of soil due to activities of burying beetles.

FIGS. 3–7. Tumble-bugs: 3, *Canthon laevis;* 4, *C. vigilans;* 5, *C. granulifer;* 6, *C. depressipennis;* 7, *C. probus.* (Natural size)

FIGS. 8–15. Dung-burying beetles: 8, *Onthophagus pennsylvanicus;* 9, *O. hecate* (male and female); 10, *Ateuchus lecontei;* 11, *Copris minutus;* 12, *Phanaeus vindex* (male and female); 13, *Ph. igneus* (male and female); 14, *Geotrupes egeriei;* 15, *Pinotus carolinus.* (Natural size)

FIGS. 16–21. Activities of tumble-bug *Canthon laevis:* formation of fecal ball at edge of stool (16, 17), rolling of ball (18–20), and burial at a distance from original site (21).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BOOKS RECEIVED

Influenza, A Review of Current Research, by various authors, WHO Monograph Series #20. 224 pp. illustrated. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. French edition in preparation. 1954. Price $2.50, 17/6d., Sw. fr. 10.—

Advances in the Control of Zoonoses. Bovine Tuberculosis, Brucellosis, Leptospirosis, Q Fever, Rabies. WHO/FAO Seminar on Zoonoses, Vienna, November 1952. WHO Monograph Series #19. 276 pp. illustrated. Distributed by International Documents Service, 2960 Broadway, New York 27, N. Y. French edition in preparation. Also published as FAO Agricultural Studies #25. Price $3., 15/—, Fr. fr. 960.—, Sw. fr. 12.—

Joint FAO/WHO Expert Committee on Nutrition. Third report. FAO/WHO Technical report series #72. 30 pp. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. Price $.20, 1/6, Sw. fr. 0.80.

Salmonellae and Shigellae. Laboratory diagnosis correlated with clinical manifestations and epidemiology, by Alfred J. Weil, M.D., The Bronx Hospital, New York and Ivan Saphra, M.D., The Beth Israel Hospital, New York. 252 pp., 29 illustrations. Chas. C. Thomas, Springfield, Ill. 1953. Price $7.75.

Gesundheitsbüchlein für die Tropen, by Dr. Ernst von Haller, Lower Buchanan, Grand Bassa Co., Liberia., 92 pp., paper covers. Stuttgart, O., Georg Thieme Verlag, 1951. Price DM 5.70.

Global Epidemiology. A geography of disease and sanitation. Vol. 3. The Near and Middle East, by James Stevens Simmons, Dean, Harvard University School of Public Health; Tom F. Whayne, Colonel, M.C., U. S. Army; Gaylord W. Anderson, Director, School of Public Health, University of Minnesota; Harold Maclachlan Horack, Instructor, Tulane University School of Medicine. Associate Author, Ruth Alida Thomas, and Collaborators. 357 pp. New York, J. P. Lippincott Co., 1954. Price $12.00.

Eleven Blue Men . . . and other narratives of medical detection, by Berton Roueché. 215 pp. Boston, Mass., Little Brown & Co., 1954. Price $3.50.

An Introduction to Pathology and Bacteriology for Medical Students in the Tropics, by the late E. C. Smith, Revised by R. Kirk. 2nd edition, 390 pp. illustrated in color. New York, Staples Press, 1954. Price $9.00.

An Rh-Hr Syllabus. The Types and Their Applications. Modern Medical Monographs, 9, by Alexander S. Wiener, Senior Bacteriologist (Serology) to the Office of the Chief Medical Examiner of New York City. 82 pp. illustrated. New York, Grune & Stratton, 1954. Price $3.75.

Rh-Hr Blood Types. Applications in clinical and legal medicine and anthropology, by Alexander S. Wiener, Senior Bacteriologist (Serology) to the Office of the Chief Medical Examiner of New York City. 763 pp., illustrated. New York, Grune & Stratton, 1954. Price $11.50.

BOOK REVIEWS

Traité de Zoologie. Anatomie, Systématique, Biologie. Tome I, Fascicule II, Protozoaires: Rhizopodes, Actinopodes, Sporozoaires, Cnidosporidies. Publié sous la direction de M. PIERRE-P. GRASSÉ, Membre de l'Institut, Professeur à la Sorbonne (Paris), avec 9 collaborateurs. 1,160 pp. 883 illustrations, 2 in color. Paris (6e): Masson & Cie., 120 Boul. Saint-Germain. 1953. Price: paper, 9,215 fr.; boards, 9,935 fr.

The publication under review is bound separately as the second part of Volume I of a comprehensive system of zoölogy prepared by a large group of distinguished French investigators. The work as a whole and in each division of the Animal Kingdom is designed to include anatomic, systematic and biologic data. Part I of Volume I provides a general introduction, a special introduction to the Protozoa and a consideration of the flagellate protozoa. Part II of Volume I presents the super-classes Rhizopoda, Actinopoda, Sporozoa and Cnidosporidia. Volume II (in preparation) will consider the Ciliata and "the cell."

The Rhizopoda are divided into three classes, Lobosa, Filosa and Granulo-reticulosa (based on the classification of De Saedeleer, 1934), although it is admitted by the editor-in-chief that these groupings, particularly the third one, are not wholly satisfactory. The Lobosa comprehend the Amoebaea, which have a naked protoplast, and the Testacealobosa, which are provided with a theca or shell. The chapter on the Amoebaea (true amebas) was contributed by the late Professor E. Chatton, and has been supplemented by the editor-in-chief. Following a presentation of the morphologic and biologic characteristics of the common free-living species *Chaos diffluens* (syn. *Amoeba proteus*), there is a description of different physionomic types, with particular emphasis on the nucleus and its methods of mitosis, nutritional requirements, encystation, the phenomena of parasitism and symbiontism in certain species, and life cycles. This is followed by a systematic classification and description of the better recognized genera and species. *Dientamoeba* and *Histomonas* are considered to be close relatives, and are placed in a separate family from the genera *Endamoeba*, *Entamoeba* and *Endolimax*, while *Pseudolimax* (= *Iodamoeba* Dobell, 1919) is assigned to the category "Sedes Incertae." Considering the comprehensiveness of the treatise, adequate information is provided concerning species of amebas of special interest to the parasitologist.

In the space available for this review it is not possible to include expansive comment on each group of protozoa presented in the volume. However, the following deserve special mention: the chapter on the ordinal groups Testacealobosa and Testaceafilosa, comprehending the Rhizopoda Testacea or thecamebas (auct.), prepared by Doctor Georges Deflandre, and including a magnificent color plate; the chapter on the Foraminifera, by Professor Jean le Calvez, and three chapters on the Actinopoda, including the class groups Acantharia, Radiolaria and Heliozoa, by Doctor G. Trégouboff. All of these are praiseworthy contributions.

Lengthy treatment is given to the superclass Sporozoa, with one chapter each on the Gregarinomorpha n. nov., Coccidiomorpha and the suborder Haemosporidia (of the order Eimeriidea), all of which have been prepared by the editor-in-chief. Of special note to students of the coccidia is the remark (bottom p. 768) that "l'*Isospora* des Limaces est trop mal connue pour être prise comme espèce type des Diplosporidae et mise en synonymie avec des Diplospora des Vertébrés. En outre, il y a toute chance pour que le parasite de la Limace appartienne à un autre genre que les Haloëimeriidea disporées des Vertébrés." Hence the species *belli* and *bigemina* are referred to the genus *Diplospora*. No mention is made of the species *hominis* in the systematic section, although *D. hominis* is referred to earlier (p. 725) where *belli* is not listed. Ample space is given to the family Plasmodiidae, with very satisfactory presentation of all aspects of the life cycles of members of this group. Sporozoa of uncertain classification, having characteristics in part like *Haemoproteus* and in part like *Plasmodium*, are grouped in a new superfamily, Babesioidea and are treated in a separate

chapter by Professor Raymond Poisson. Shorter chapters are devoted to the Sarcosporidia and the Haplosporidia.

Parasitic protista, intra-cellular or extra-cellular, of uncertain status, including among other genera, *Grahamella*, *Bartonella*, *Toxoplasma* and *Rickettsia*, are considered as a group. This is one of the few chapters which microbiologists will criticize, partly because some of the genera included are unrelated to one another and partly because some of them do not belong to the protozoa. Similarly the earlier classification of the Acrasias, Mycetozoa (syn. Myxomycetes) and Plasmiophorales among the protozoa will be challenged by the mycologists.

The superclass Cnidosporidia, contributed by Professor Poisson, completes Vol. I, Part II.

The balance of subject matter as a whole and within separate sections is admirable in view of the vast amount of material which has required coverage. The authors have included recent information, for example, mention of fumagillin as an antiamebic agent. Moreover, their literature citations at the end of each chapter or group of chapters have a cosmopolitan coverage, although the reviewer would have utilized some unlisted references as more representative and more significant.

The style is lucid, the contents easy to read and to interpret. The illustrations are abundant, of superior artistry and reproduction. There is a comprehensive subject index at the end of the volume. The format is pleasing and the paper well selected to reproduce clearly both textual material and illustrations. Few typographic errors have been encountered, mostly in connection with authors' names. The volume is handsomely bound.

Some readers will take exception to portions of the systematic classification, but the material as a unit constitutes a distinct achievement, the only recent one of its kind in any language. It deserves a place as a reference text in the libraries of zoölogists, protozoölogists and parasitologists throughout the world.

ERNEST CARROLL FAUST

Clinical Tropical Diseases, by A. R. D. ADAMS and B. G. MAEGRAITH, from the School of Tropical Medicine, Liverpool. 508 pp. illustrated. Springfield, Illinois: Charles C Thomas, 1953. Price $10.50.

The avowed purpose of this new handbook is to provide that "information essential for the clinical diagnosis and the treatment of those diseases which occur primarily in the tropics." Essential facts are supplied "as dogmatically and concisely" as possible. None other than "the most brief descriptions" are presented of the causative organisms or of the responsible vectors as "such information is readily available in the textbooks on bacteriology and parasitology." To achieve this objective, the subject matter is presented in a series of 47 short chapters, which are organized on an alphabetical rather than etiological basis. No references are cited. The format is pleasing, and the point is made that the book is bound in insect-proof cloth.

In general, the stated objectives are achieved with the presentation of brief clinical summaries and resumés of accepted methods of treatment. Yet, not infrequently, statements are made that appear to the reviewer to be unduly dogmatic and to require qualification. For example, on page 7, in discussing amebiasis it is stated that "asymptomatic intestinal infections are the rule in those acquiring their infections in temperate climates," and on page 248 with reference to whipworms, "the presence of the latter (adult worms in the intestine) causes no symptoms or gross pathology."

It would appear that the authors assume that this book will be used by workers who also possess reference books on the laboratory aspects of parasitic infections or else have available qualified assistants in the laboratory. In the section on amebiasis the diagnosis is stated to rest on the identification of the causative parasite, yet no mention is made of the nonpathogenic amebae or of the need for differentiation of these forms. The one plate (Fig. 39) which depicts helminth eggs and cestode morphology does not approach in quality the clinical illustrations in the text. The usefulness of this volume might be augmented in subsequent editions by moderate expansion of those portions dealing with laboratory diagnosis.

The blank space at the end of certain of the chapters could well be utilized to present the reader with a few key references.

THOMAS H. WELLER

A Guide to Human Parasitology for Medical Practitioners, by D. B. BLACKLOCK, C.M.G., M.D. (Edin.); D.P.H. (London); D.T.M. (Liver.) and T. SOUTHWELL, D.Sc., Ph.D., A.R.C.Sc., F.Z.S., F.R.S.E.; revised by T. H. DAVEY, O.B.E., M.D. (Belf.); D.T.M. (Liver.). 5th edition. 228 pp., 3 plates in color and 120 text illustrations. Baltimore: The Williams & Wilkins Co., 1953. Price $5.50.

From its first appearance in 1931, this book has been intended primarily for the practitioner who has at his disposal only ordinary laboratory facilities for making a diagnosis in diseases caused by animal parasites. It is pleasant to note from the copyright page that previous editions have been reprinted seven times; a truly excellent testimony of its past cordial reception. Although somewhat smaller than the previous edition, discussion of some protozoa and helminths previously omitted or briefly mentioned are now included or considered in greater detail. It is unfortunate, however, that some increasingly important parasitic infections and diseases, such as swimmer's itch and toxoplasmosis, are not included in the subject matter, whereas valuable space is given over to such rare helminth infections in man as fascioliasis, and, Chapter V, a total of 12 pages, is devoted to the spirochetes.

Despite the innovation of a short paragraph on prevention concerned with each parasite, the book, for the practitioner's need, is not well balanced. It is strong on the biological phases of the parasite but weak on the clinical and public health aspects. Chemotherapy, in this volume, is sometimes mentioned, but recommendations for specific therapy and a résumé of new drugs for treatment and for prophylaxis—information particularly needed by the practioner inexperienced in parasitology—are not made. For such information he will be obliged to resort to time-consuming search through periodic literature or larger text-books. A list of such books was previously included, but this helpful feature has been omitted in the present volume.

This book is singularly free from typographical errors, and descriptions are clear. The color plates are excellent, the print is easily read, and the book supplies a good elementary coverage of human parasitology.

DONALD L. AUGUSTINE

Textbook of Public Health (formerly Hope and Stallybrass), by W. M. FRAZER, M. D., D.P.H., Professor of Public Health, University of Liverpool; 13th edition, 663 pp., 76 illustrations. Baltimore: The Williams & Wilkins Co., and Edinburgh & London; E. & S. Livingstone, Ltd., 1953. Price $8.50.

This is a comprehensive, almost encyclopedic, text covering every field of public health. The author is a lawyer as well as a doctor, and the legal influence is strongly apparent in the detailed description of laws in England relating to the various fields of public health covered in the text. There are excellent descriptions of the organization of all levels of public health activity in Great Britain and the legislation pertinent to this organization. There is a clear description of the National Health Service and its interrelationship with the public health authorities. From this content alone the book is of value to American students of public health as a clear and concise description of the administrative features of public health in Great Britain. As a result of the extensive and comprehensive nature of the book the descriptions of all features of public health are necessarily brief and not as detailed in some cases as one might desire. However, all sections are clear and to the point and give a good coverage of most features. Due to the heavy emphasis on British aspects of public health this book would probably not be useful as a general textbook in the United States. However, due to its comprehensive nature it would appear to be a must for most libraries as a general source book for the student of Preventive Medicine and Public Health. It would be equally useful as a reference book in departments of public health.

JOHN M. WEIR

Acute Infectious Hemorrhagic Fevers and Mycotoxicoses in the Union of Soviet Socialist Republics. Army Medical Service Graduate School and Walter Reed Army Medical Center. 140 pp., Washington 25, D. C., U. S. Government Printing Office, May 1953. Price $.60.

In 1951 the U. N. Forces in Korea encountered an acute infectious disease with hemorrhagic manifestations which had already been reported by the Japanese and Russians in the basin of the Amur River. This epidemic disease is only one of a number of similar but distinct hemorrhagic fevers which occur in various parts of the Soviet Union and are transmitted by an arthropod vector. This monograph, the second of a new series of publications by the Army Medical Service Graduate School, performs the useful and timely service of summarizing the Russian papers on the subject which have reached America. The literature also referred to certain toxicoses caused by food contaminated with molds and presenting clinical manifestations resembling the hemorrhagic fevers. Since information on these mycotoxicoses is lacking in English, a portion of the monograph is devoted to this subject.

L. W. HACKETT

The Housefly, by LUTHER S. WEST, Comstock Publishing Company, Inc., Ithaca, New York, 1951, 584 pp., 176 figures. Price $7.50.

The foreword by Col. Paul Russell enumerates some of the serious diseases in which the housefly is concerned. These important disease problems are treated from a historical and practical viewpoint in Chapters 1, 11, 12, and 13 of this Volume of 19 chapters.

The life history, habits, morphology, physiology, ecology and distribution of the housefly and its relatives are discussed, and the parasites, pedators, symbionts, and commensals are briefly considered.

The fly as an experimental animal, including its use in insecticide testing, and the technics involved in such work are discussed. The treatment of field, museum, and especially laboratory technics go far beyond the housefly. This is also true of the chapter on myiasis, which deals mainly with flies other than those of the genus *Musca*.

Emergency and planned control, and use of the newer insecticides are satisfactorily covered. It is noted that a tent-type fly trap is illustrated (Figure 146). This type is definitely inferior to traps of cone-type.

The gleaning of the important facts from the widely scattered literature on a subject so much investigated and written about is a herculean task. Overemphasis is placed on the size of the genus *Musca* by the long list of species in Table 4 (pp. 136–144). It would have been better to group the synonyms under their proper names. Of the 204 forms listed, at least 29 are misidentifications or erroneous spellings.

No exhaustive work on the housefly has appeared since the splendid books of L. O. Howard (1911) and G. Gordon Hewitt (1914) were published. During this period tremendous advances have been made in our knowledge of the housefly—man's constant companion and deadly enemy. The appearance of Dr. West's exhaustive review of these advances is therefore timely and will be widely used.

F. C. BISHOPP

World Directory of Medical Schools, WHO. 220 pp., clothbound, bilingual edition, English-French. Geneva, 1953. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $4., 25/-, Fr. Fr. 1,200. Sw. fr. 15.

More than 500 medical teaching institutions are listed in this Directory in tabular form, with columns indicating date of founding, public or private nature, academic year, conditions of admission, number of teachers and students, number admitted and graduated each year, degrees conferred, language of instruction, length of course and tuition fees. The data were collected from the institutions themselves in most cases. New editions are planned at

intervals to correct and bring up to date the information presented. Unfortunately the 20 pages devoted to the Union of Soviet Socialist Republics, while listing the institutions of medical education, are completely blank as regards all the information listed above, and of the satellite countries we know almost as little. Otherwise, the directory is surprisingly comprehensive and will have many uses.

L. W. HACKETT

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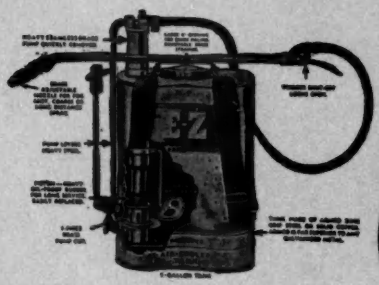

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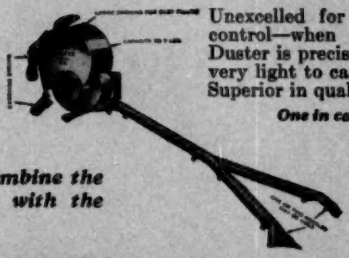

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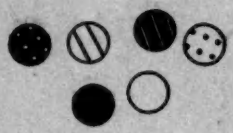
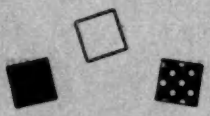
BACTO-SENSITIVITY DISKS

for Antibiotics



for Sulfonamides

for Other Therapeutic Agents



Bacto-Sensitivity Disks permit the determination of the sensitivity of microorganisms to antibiotics, sulfonamides and other chemotherapeutic agents including the anti-tubercular compounds.

Bacto-Sensitivity Disks are sterile and adhere to the inoculated medium without crumbling or slipping, permitting rapid uniform diffusion. They are prepared in three concentrations of each therapeutic agent, designated by markings on the disks; the color of the disk indicates the agent. The three concentrations make it possible to determine if an organism is sensitive, moderately sensitive or resistant. Sensitivities of most microorganisms are determined by overnight incubation at 35-37°C.; tubercle bacilli sensitivities may be determined in as short a period as 6-10 days.

The value of Bacto-Sensitivity Disks has been fully demonstrated by continued use under practical conditions. They are especially valuable in selecting the therapeutic agents effective against persistent infections refractory to primary therapy. The reliability and ease of the disk plate method for determining the resistance or susceptibility of microbes to the various therapeutic agents makes the use of Bacto-Sensitivity Disks highly practical in clinical and diagnostic laboratories.

Booklet No. 137 describes Bacto-Sensitivity Disks and explains how to use them. A copy will be sent upon request.

DIFCO LABORATORIES

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